Investigating the effect of uterine artery embolisation on the uterus of women with fibroids and heavy menstrual bleeding

Ibraheem Hamoodi
MBChB MRCOG

Submitted to the University of Glasgow in fulfillment of the requirements for the degree of Medical Doctorate

Obstetrics and Gynecology
Medicine Graduate School
College of Medical, Veterinary and life Sciences,
University of Glasgow
April 2017

Abstract

Introduction:

Uterine fibroids are common benign tumours in women. The effect they have can range from pressure symptoms and heavy menstrual bleeding to denying a woman a successful pregnancy. Despite the overall benign classification of fibroid, it is undeniable that the reduced quality of life these tumours cause may exceed the effect that some early stage gynecological cancers have on a suffering woman. There has been extensive research into treatments for fibroids however this has only resulted in a handful of worthy advances which are not proportionate to the impact these "benign" tumours have on a suffering woman. The mechanism for how fibroids affect the endometrium and cause heavy menstrual bleeding is understudied. Some of this research has pointed towards vascular endothelial growth factor and cyclooxygenase as potential mediators that change the perceived heaviness –amount and length- of a menstrual period in a woman with fibroids.

Uterine artery embolisation (UAE) has emerged as a lesser invasive option and an alternative to surgery. The reported high technical success of the procedure independent of BMI and the short hospital stay and quick recovery has made it an attractive treatment modality. However, the mechanism of how it works is still not understood. Women with large variation in uterine size and fibroid number and size are undergoing this procedure without the full knowledge of how they will individually respond.

This thesis aims to explore the mechanism of how UAE works by looking at the change in expression of VEGF, COX-2 & Ki67 and also the change in microvascular density of the endometrium post UAE. Our aim was to investigate how women with different sized fibroid uteri would respond to UAE and if DWI MRI had any advantages above standard MRI in predicting the outcome of UAE.

Methods:

In this thesis we describe the use of endometrial biopsies taken from women with fibroids and HMB before and after the UAE procedure. The expression of the markers mentioned above was measured using mainly immunohistochemistry. Standard MRI imaging & newly emerging DWMRI was used extensively in this thesis to evaluate the response to UAE.

Results:

The study found an overall trend of increased VEGF expression and increased microvascular density in the endometrium post-UAE. Proliferation of the endometrium as assessed by Ki67 expression in both the proliferative stage and early secretory stages of the endometrium seemed to increase post UAE. However there was also an overall trend of decreased expression of COX-2 post UAE. Due to low sample numbers, statistical analysis was not performed in some group comparisons.

We found around 95% of the patients had a reduction in their uterine & dominant fibroid size post UAE. Mean fibroid volume reduction ranged between 38-70% according to dominant fibroid location while a fibroid uterus reduced in volume by an average of 38%. Age of the patient, number of fibroids and the location of the fibroid all play a small role in determining the response to UAE. The complication rate of UAE is low however re-intervention rate and the need for additional treatment appear to rise with time.

Diffusion weighted MRI (DWMRI) is an understudied imaging technique. In this thesis a pilot study demonstrated that it may have a role to play in both predicting and assessing the response to UAE. The Apparent Diffusion Coefficient (ADC) values were unchanged immediately after and 6 months post UAE, indicating that normal uterine tissue –unlike fibroids-is unaffected by the ischemia-like conditions following UAE.

Using the pictorial blood loss assessment chart score (PBAC) the study demonstrated a significant reduction in menstrual blood loss. However, the score did not relate to the size of the uterus or size and location of the fibroids pre-UAE. It also did not significantly correlate to the change in volume of the above or the endometrial markers studied.

Conclusion:

It appears that the endometrial markers studied play a role in explaining the mechanism of how UAE affects the endometrium and how symptom improvement is achieved. Standard MRI and DWMRI both play an important role in assessing response to UAE.

Table of Contents

List of tables

List of figures

Acknowledgement

I would like to thank Professor Mary Ann Lumsden for her careful and kind supervision throughout the period of my research and the time of writing this thesis. I would also like to thank her for her patience and prompt reply to all my queries.

I would like to thank Professor Jonathan Moss for all that he taught me while attending his interventional radiology clinics at Gartnavel general hospital and helping me develop my research ideas and pointing me in the right direction with the radiology part of my research

I would like to thank Dr Salha Abukhnjr for her valuable advice and her laboratory expertise that helped guide me. Her help in the laboratory was invaluable and her previous work helped guide and focus my research.

I extend my sincerest gratitude to Rob Ferrier and Dr Clare Orange for allowing me access to the laboratory at Queen Elizabeth Hospital and guiding me through the immunohistochemistry process from retrieving slides from the archives and all through the different staining processes.

I am forever grateful to all the nursing staff at ward 56 at Princess Royal maternity unit & Clinic F at Stobhill Hospital who allowed me access to their relevant clinical areas and the use of their clinical rooms to recruit, assess and treat patients in my research.

I should thank Dr Sue Lassman for her one to one sessions in teaching me how to make sense of pelvic MRIs and helping me understand diffusion weighted scans which helped form the basis of one the chapters in this thesis.

I would like to dedicate this thesis to my wife Victoria for her love and unconditional support during my research period. Her encouragement pushed me through the difficult periods and her constant support & belief in me helped me complete this thesis. I thank her for looking after our very young children Rose & Jude and making up for the time I missed being with them.

Author's Declaration

Except where due acknowledgment is made by reference, the studies undertaken in this thesis were unaided work of the author. No part of this work has been previously accepted for, or is currently being submitted for another degree or professional qualification.

Dr Ibraheem Hamoodi

Abbreviations

ADC	Apparent Diffusion Coefficient
cDNA	Complementary DNA
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
CRIS	Clinical Research Information System
D&C	Dilatation and curettage
DCE-MRI	Dynamic contrast enhanced MRI
DUB	Dysfunctional uterine bleeding
DWMRI	Diffusion Weighted Magnetic Resonance Imaging
E_2	Oestradiol
ER	Oestrogen receptors
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin releasing hormone
GP	General practitioner or practice
HCG	Human chorionic gonadotrophin
HMB	Heavy menstrual bleeding
HSPG	Heparin sulfate proteoglycan
ISD	Information Services Division Scotland
IVF	In vitro fertilisation
LH	Luteinizing hormone
MBL	Measured blood loss
MRgHIFUS	Magnetic Resonance-Guided High Intensity Focused Ultrasound
MRI	Magnetic resonance imaging
NSAID	Non-steroidal anti-inflammatory drug
PA	Plasminogen activators
PACS	Picture archiving and communication system
PBAC	Pictorial Blood loss Assessment Chart
PG	Prostaglandin
PR	Progesterone receptors
R&D	Research & Development
RNA	Ribonucleic acid
SPRMs	Selective progesterone receptor modulators
Tx Acid	Tranexamic acid
UAE	Uterine artery embolisation
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor-A

1. Chapter 1: Introduction

1.1 The human uterus

The human uterus is an inverted pear shaped fibromuscular organ that is located in the pelvis in a non-pregnant state. It occupies both the pelvis and abdomen when in a pregnant state and is then known as the gravid uterus. It is composed mainly of a thick, smooth muscular outer layer known as the myometrium and a thin inner layer lined by endometrial cells known as the endometrium. This in turn surrounds the uterine cavity. There is also a further thin outer layer known as the serosa which completely covers the uterus posteriorly and only partially anteriorly. The cavity of the uterus has one opening inferiorly that leads to the uterine cervix. However, superiorly it has two openings, one on either side that lead into each fallopian tube known as the ostia. The cells in the myometrium can undergo both hypertrophic and hyperplastic changes during a woman's reproductive life. This leads to a change in the size of the uterus that is mostly noticeable at puberty and throughout pregnancy (gravid state). The endometrial layer is a dynamic layer that undergoes cyclical changes on a near monthly basis. These changes involve shedding (menstruation), regeneration and remodelling in preparation for conception. These changes occur throughout the monthly menstrual cycle and are influenced by the ovarian sex steroid hormones and are often referred to as phases. They coincide with changes that occur in the ovaries known as the ovarian cycle (fig 1-1).

The first day of the menstrual cycle is the day of menstrual shedding which signals the end of one cycle and the start of another. The menstrual phase usually lasts 5 days (+/-2) where both oestradiol and progesterone are at the lowest level. Following this phase, there is a rise in the level of oestradiol leading to the proliferative phase. In this phase the endometrium grows and thickens (proliferates). Ovulation occurs mid-cycle (usually day 14) and progesterone is then produced from the corpus luteum and the cycle moves into the secretory phase. In this phase the dominant sex steroid is progesterone which in association with oestradiol prepares the endometrium and renders it suitable for implantation of the fertilised ovum and the start of a pregnancy. In the vast majority of times implantation does not occur. This leads to a drop in the progesterone level secondary to corpus luteal regression and menstruation occurs.

1.2 The endometrium

1.2.1 Endometrial structure and the menstrual cycle

The endometrium consists of two layers or zones. The first layer is the superficial functioning zone that is shed during each menstruation. The second layer is known as the deep basilar zone that is not shed and from which the endometrium regenerates. The endometrium undergoes three phases, the proliferative phase, the secretory phase and the menstrual phase, which is collectively called the menstrual cycle (Fig 1-1).

1.2.1.1 Proliferative phase

It is the stage of the menstrual cycle in which the ovarian follicles prime themselves for ovulation. It is also known as the pre-ovulatory phase of the menstrual cycle. In this phase the rebuilding process of the endometrium starts. The functional zone is shed during menses leaving the basilar zone exposed. The surface epithelial cells regenerate and the epithelial glands gradually elongate and curve. The stroma is dense, containing spindle shaped cells and stromal oedema tends to regress through the proliferative phase. These proliferative changes are more prominent in the functionalis layer than the basalis layer. The biologic rationale for the environmental variation in proliferative indices may lie in the different physiologic functions of the functionalis layer versus the basalis layer. Where the functionalis layer is the site of blastocyst implantation, the basalis layer provides the origin for regeneration of the endometrium. Although oestradiol (E_2) is the prominent sex hormone in the pre-ovulatory period, many authors demonstrated that both intracellular oestrogen receptor (ER) and progesterone receptor (PR) concentrations are at their highest during this period of the menstrual cycle [1-3]. This is in keeping with the hypothesis that PR synthesis is mainly induced by E_2 in the target cells via the E_2-receptor complex mechanism. Therefore, the presence of PR in the proliferative phase is a good indicator of endometrial E_2 sensitivity.

1.2.1.2 Secretory phase

Once ovulation occurs, the oestrogen-primed endometrium undergoes changes under the influence of progesterone. Progesterone inhibits the proliferative activity of the endometrium and induces a complex secretory activity that starts with the polarization of glycogen at a subnuclear location followed by its transport via microfilaments to the apical region of the cell. Secretory granules are formed when the Golgi apparatus packages the glycogen and various other substances. These granules are then expelled into the glandular lumen. This period is known as the secretory phase and the daily changes are specific to post-ovulatory endometrium. These changes are used in dating the endometrium as the structural changes are apparent on routine examination and can indicate whether ovulation has or hasn't taken place. In an average cycle the secretory phase duration is constant whereas the length of the follicular phase is variable. This phase of the endometrium is further subdivided into three stages; early, mid and late secretory.

1.2.1.2.1 Early secretory stage

This stage represents 1-4 days post-ovulation (day 15-18 of the cycle). During this stage the epithelial gland cells acquire subnuclear glycogen vacuoles and the nuclei lose their pseudostratification configuration. The glands exhibit a regular tortuosity and are clearly oriented from the base to the surface of the endometrium.

1.2.1.2.2 Mid-Secretory stage

This stage represents 5-8 days post-ovulation (day 19-23 of the cycle). During this stage there is maximum intra-glandular secretion which coincides with blastocyst implantation. There is maximal stromal oedema secondary to transudation of plasma from endometrial blood vessels. The spiral arterioles become more prominent due to thickening of their walls, coiling and endometrial proliferation.

1.2.1.2.3 Late secretory stage

This stage represents around 9 days post ovulation and lasts until menstruation (day 24 of the cycle to the menstrual phase of the next cycle). Peri-vascular pre-

decidulalization takes place which consists of cytonuclear enlargement with mitotic activity and the formation of a pericellualr laminin-rich basement membrane particularly of the epithelial cells [4]. The spiral arterioles are surrounded by predecidual stroma and there is stromal regression and loss of endometrial height.

1.2.1.3 Menstrual phase

In the absence of conception, there is progesterone withdrawal. This is secondary to human chorionic gonadotrophin (HCG) not being produced by the newly formed placenta and therefore the corpus luteum is unable to sustain itself. The gland secretion is exhausted and involution of the epithelial glands and stromal collapse ensues leading to shedding of the functionalis layer. The menstrual tissue that is expelled vaginally is the result of the enzymatic auto-digestion and prostaglandin related ischemic necrosis of non-gestational oestrogen/progesterone primed endometrium [5]. The remaining basalis layer and the deep functionalis layer of the endometrium begin the regeneration process at day three of this phase.

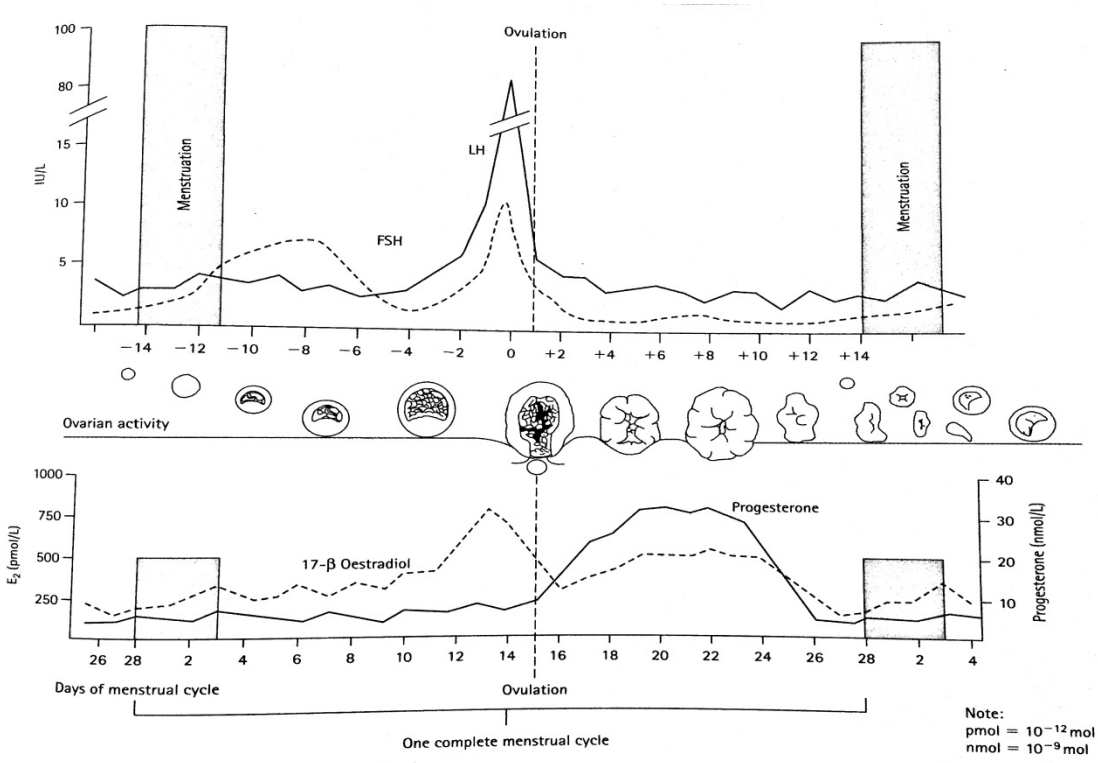


Figure 1-1: The menstrual cycle

1.2.2 **The endometrial blood supply**

The uterus is supplied by two main vessels known as the uterine arteries. These usually arise from the anterior division of the internal iliac artery that is a branch of the common iliac artery that arises from the bifurcation of the aorta at the level of the fourth lumbar vertebrae (L4). The blood supply of the endometrium comes from branches of the circumferentially oriented arcuate arteries in the myometrium that are derived from the uterine arteries. These branches form straight arteries which supply the basalis layer and spiral arteries which supply the functionalis layer. The spiral shaped arteries are unique to menstruating species [6]. As discussed earlier these blood vessels undergo cyclical changes as part of preparing the endometrium for pregnancy and like in the heart are ‘end arterioles’.

Endometrial angiogenesis is likely to occur through a process of elongation and expansion of pre-existing blood vessels [7]. Unlike most vascular beds that keep a persistent structure throughout life, the endometrial blood vessels grow and regress during the menstrual cycle and are therefore thought to be controlled by the circulating oestrogen and progesterone [8]. The growth of these vessels must then be mediated by the presence of oestrogen receptors (ER) and progesterone receptors (PR) in the endothelium of these vessels. However, there are conflicting reports on the expression of ER and PR in human endometrial endothelium both in vivo and in vitro [9, 10]. It may be that there is an indirect effect of these hormones on the blood vessels. One study showed that this may be mediated by certain components of the basement membrane such as heparan sulfate proteoglycan (HSPG). The disappearance of HSPG from the vascular basement membranes may play a role in the vascular remodelling process during the menstrual phase [11]. Progesterone withdrawal seems to induce significant elevations in fibronectin. There is a correlation between the repair of the endometrium and the expression of fibronectin and its receptors after menstruation [12].

1.3 Steroid hormones and their receptors in the endometrium

Oestrogen and progesterone play a significant role in the cyclical endometrial changes that occur throughout the menstrual cycle. The variation in the concentration of these ovarian sex steroid hormones control these changes [13]. The appropriate response to increasing and decreasing hormone concentration requires dedicated receptors. The transcriptional effects of oestradiol and progesterone involve classical nuclear receptors (ER and PR). These, together with androgen receptors and glucocorticoid receptors belong to the nuclear receptors subfamily 3 group shuttling between the cytoplasm and the nucleus. PR and ER have also been shown to mediate rapid activation of non-genomic signalling pathways independent of their transcriptional activity and alternative membrane receptors may be involved in this [14].

1.4 Vascular Endothelial growth Factor (VEGF)

It is an important angiogenic cytokine with a critical role in tumour angiogenesis [15]. Under low oxygen conditions the endothelial cells produce vascular endothelial growth factor (VEGF) as it has a role in enhancing the mitosis of endothelial cells as well as their permeability. It exerts its effect by binding to two types of tyrosine kinase receptors on the endothelial cells [16]. The first is known as VGEF receptor 1, which is responsible of the organization of endothelial cells in vessels and the second being VGEF receptor 2, which induces endothelial cell migration and proliferation. Both receptors are upregulated in a hypoxic environment (further details in section 3.1)

1.5 Prostaglandins & Cyclooxygenase

Prostaglandins (PGs) are lipid autocoids derived from arachidonic acid. They sustain homeostatic functions and mediate pathogenic mechanisms, including the inflammatory response. They modulate biological functions in several systems in the body including the cardiovascular, gastrointestinal, genitourinary,

endocrine, central nervous system, immune, respiratory and reproductive systems. PGs were first observed in 1930 by two gynaecologists Kurzrok and Leib while studying the effect of the seminal fluid on the human uterus. It was noted then that they reduced blood pressure and caused smooth muscle contraction. At that time it was thought that they were produced by the prostate and hence the name prostanoids. They were successfully extracted and purified in 1957 and were later subdivided alphabetically according to the substituent groups on their cyclopentane ring.

PGs are formed when arachidonic acid (AA), a 20-carbon unsaturated fatty acid, is released from the plasma membrane by phospholipases. PG production (figure 1.3) depends on the activity of PGG/H synthases, colloquially known as cyclooxygenases (COXs), bi-functional enzymes that contain both COX and peroxidase activity and that exist as distinct isoforms referred to as COX-1 and COX-2 [17]. PGH2 is produced by both COX isoforms, and it is the common substrate for a series of specific isomerase and synthase enzymes that produce PGE2, PGI2, PGD2, PGF2α, and TXA2. COX-1 couples preferentially, but not exclusively, with thromboxane synthase, PGF synthase, and the cytosol (c) PGE synthase (PGES) isozymes [18].

The biological action of prostaglandin is mediated through their receptors, which are designated by the letter P and a prefix D, E, F, I and T, and have the form DP, EP, FP, IP and TP referring to PGD, PGE, PGF, PGI and TXA_2 respectively. PGE2 exerts its effect through four subtype receptors, EP1, EP2, EP3 and EP4. These receptors are G-protein coupled receptors using alternate and sometimes opposing intracellular pathways. These receptors are represented with a separate encoded gene, with extra splice variant for EP3,FP and TP which are different in their C-terminal [19].

In addition to the prostanoids that act principally via plasma membrane-derived G-protein-coupled receptors, several COX products can activate nuclear receptors of specific classes, and stimulate the common nuclear receptor pathway [20]. It was found that these nuclear-acting prostanoid ligands inhibit the IkappaB kinase activity and thereby block the NF-kappaB transcription factor pathway and raise the possibility that the COX pathway may induce anti-

angiogenic effects by nuclear-acting prostanoids [21]. Therefore, a number of COX-2-selective inhibitors are developed to control the anti-inflammatory and anti-neoplastic activities of the COX-2 isoenzyme. Inhibition of the COX isoenzyme activity and/or expression may be the basis of future development of anti-inflammatory and anti-neoplastic drugs [22].

Towards the end of the menstrual cycle COX-2 expression is upregulated in parallel with the withdrawal of progesterone and shedding of the functionalis layer of the endometrium with subsequent synthesis of PGE2 [23, 24]. Excessive expression of COX-2 and PGE2 synthesis in the endometrium has been recognised in women suffering from heavy menstrual bleeding and their signalling pathways are potential therapeutic targets [25]. (Further details in section 3.2)

1.6 Endometrial angiogenesis

Angiogenesis is the process by which new microvessels develop from existing blood vessels. Normal physiological angiogenesis occurs during foetal growth and development. It rarely occurs in adults except in wound healing and in the female reproductive tract. In the female of reproductive age, angiogenesis occurs in the corpus luteum and the endometrium during the menstrual cycle [26]. Such a process is of utmost importance in the development and differentiation of the human endometrium which is necessary for implantation and maintenance of pregnancy [27]. Pathological angiogenesis occurs in conditions such as malignancy, chronic inflammatory disorders, diabetic retinopathy, endometriosis and in fibroid tumours [28].

The demand for vessel growth and regression in human endometrium differs spatially and temporally during the sequence of events that occur during the menstrual cycle. Spatially angiogenesis occurs in different regions of the endometrium according to the stage of the menstrual cycle. When menstruation is ongoing angiogenesis occurs in the superficial part of the remaining basalis layer. During the rapid endometrial growth of the proliferative phase it occurs throughout the functionalis layer under the influence of oestrogen. While during the secretory phase the growth of spiral arterioles also occurs in the functionalis layer, whereas the capillary plexus which is supplied by the arterioles develops just below the luminal epithelial surface. Vasoconstriction of the distal segments of the spiral arteries occurs in response to progesterone withdrawal. A few days later this is followed by diffuse necrosis, inflammation and vascular thrombosis resulting in endometrial shedding and menstruation.

Studies have demonstrated the importance of smooth muscle cells together with endothelial cells in the process of developing new blood vessels and the signalling that occurs between them during development [29, 30]. It is thought that proliferation of smooth muscle cells is intrinsically central to angiogenesis (figure 1-5).

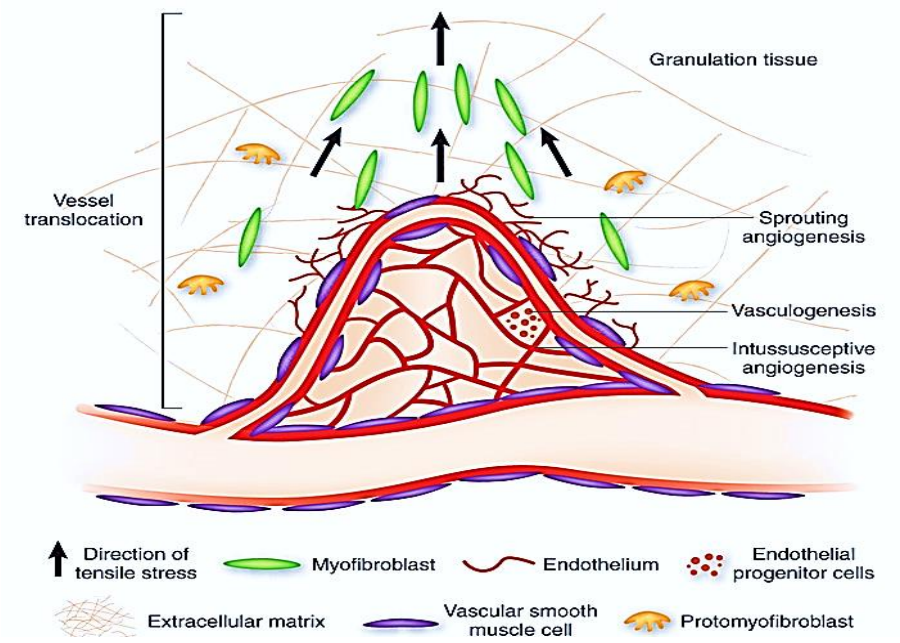


Figure 1-2: The mechanism of angiogenesis

1.7 The mechanism of normal menstruation

Menstruation in principle is the event of shedding of the superficial (functionalis) layer of the endometrium. A series of sequential events occur before, during and after this important event that occurs in a women of reproductive age on a near monthly basis. Inflammatory pathways play a role in the female reproductive health and regulate the menstrual cycle. Alterations in these pathways contribute to the pathologies of the female reproductive system [31]. Sex steroids influence endometrial growth, differentiation and function, and are also implicated in endometrium pathology [32].

Menstruation is a result of the profound tissue remodelling that occurs each month in reproductive-aged women. Regular, pulsatile secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus is the primary event that drives the mechanism of menstruation. In response to this secretion, the pituitary gland releases follicular stimulating hormone (FSH) and luteinizing hormone (LH). FSH is the principal regulator of follicular growth and maturation. It initiates follicular growth and the secretion of oestrogens. Ovulation is caused by a peak level of LH, but it is dependent on progesterone levels. LH promotes the formation of the corpus luteum and stimulates the production of oestrogen, progesterone and inhibin which in turn form a negative feedback loop on the hypothalamus (figure 1.6).
Synthesis of progesterone by the corpus luteum precedes the negative feedback of progesterone at the hypothalamic level. While at the endometrial level (the functionalis layer) the number of sex steroid receptors increases during the proliferative phase and decreases during the secretory phase.
In the absence of pregnancy, the corpus luteum collapses leading to a sharp decline in the ovarian steroids hormones. This is turn triggers compound events within the functionalis layer of endometrium leading to endometrium break down.
In 1978 JE Markee transplanted a fragment of human endometrium into the anterior chamber (iris) of rhesus monkey's eyes and his direct observations provided the initial understanding of the mechanism of menstruation [33]. The development of the implanted graft involved progressive episodes including, increased coiling of spiral arterioles, vascular stasis, alternation of

vasoconstriction and vasodilatation episodes and perivascular bleeding. Marked regression of the developed grafts was noted after oestrogen and progesterone withdrawal.

The expression of prostaglandins in many compartments of the endometrium varies across the menstrual cycle and tends to increase during menstruation. For instance, prostaglandin F_2 αlpha ($PGF_2\alpha$) causes vasoconstriction of blood vessels and leads to reduction in the blood flow to the endometrium [34]. $PGF_2\alpha$ is upregulated during the mid-secretory phase but still has high expression during menstruation. On the other hand, prostaglandin E_2 (PGE_2) is responsible for vasodilatation and is mainly increased during menstruation with a lower concentration than $PGF_2\alpha$ [35].

Upregulation of prostaglandin is associated with increased expression of locally acting mediators such as interleukins, angiogenic factors and protease enzymes. These mediators play a role in releasing and activating cytokines which in turn allows leukocyte infiltration of the endometrium [36].

Due to the above processes, it is fair to describe the mechanism of menstruation as an inflammatory process under the control of ovarian sex steroid hormones [31].

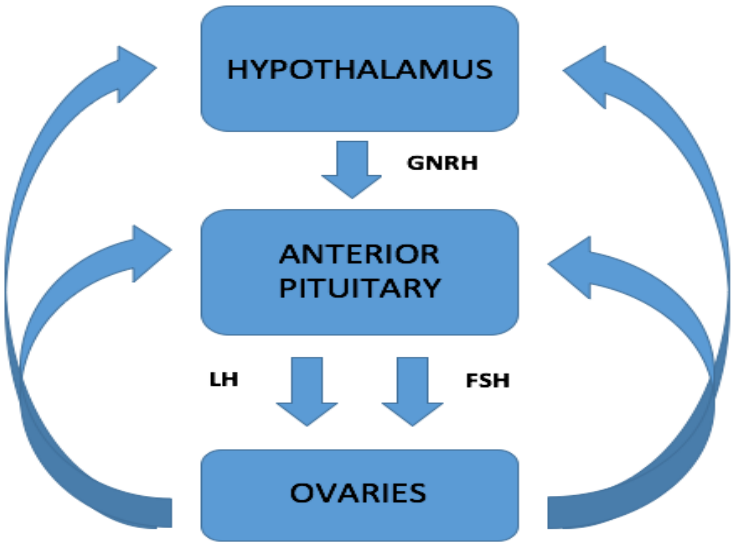


Figure 1-3: The Hypothalamic-Pituitary axis

1.8 Heavy Menstrual Bleeding

Heavy menstrual bleeding (HMB), formally known as menorrhagia is defined as excessive menstrual blood loss that interferes with a woman's physical, emotional and social quality of life [37]. HMB is considered to be one of the most significant causes of ill health in women. In the UK one in 20 women of reproductive age consults their general practitioner each year because of heavy menstrual bleeding. It accounts for 20% of gynaecology outpatient referrals making it is the most common condition that requires referral to a gynaecologist [38, 39]. In a Scottish postal survey about self-reported symptoms of heavy periods, 30% of women who were asked reported heavy menstrual loss and a further 5% reported very heavy menstrual loss. [38].

In 1966 *Hallberg et al* measured the haemoglobin level in menstruating women. He found that the average volume of menstrual blood loss per cycle was 40 ml. When the blood loss was more than 60ml, the incidence of anaemia increased [40]. Clinically, around 50% of women complaining of HMB who have a normal uterus, have objectively measured blood loss of 80ml or less [41]. Different methods have been studied to quantify menstrual blood loss and this will be discussed in some depth later in chapter 6.

It is generally accepted that menstruation is associated with an element of pain. This is usually described as cramps, backache and pelvic pain. A UK-wide national audit concluded that 54% of pre-menopausal women reported pain. This was higher in the presence of uterine fibroids and endometriosis. However, severe pain seems to be one of the main symptoms that accompanies HMB and is generally reported in up to 15% of women[42].

HMB should be recognised as having a major impact on a woman's quality of life, and interventions should be aimed at improving it and not just focusing on reducing menstrual blood loss alone.

1.8.1 **Mechanisms leading to HMB in the normal uterus**

Idiopathic HMB also called dysfunctional uterine bleeding (DUB) is defined as HMB in the absence of microscopic and/or macroscopic uterine pathology or pregnancy [43]. The vast majority of women with DUB will have normal endometrium and a minority of young women will have a hereditary coagulation abnormality such as Von Willebrand [44]. Progesterone inhibits plasminogen activators (PA) which is reflected by reciprocal upregulation in the output of the PA inhibitor PAI-1 [45]. High levels of circulating PAI-1 are associated with a number of thrombotic diseases, however, PAI-1 deficiency can have the opposite effect and lead to HMB [46].

Twin studies found a hereditary link between menstrual blood loss in monozygotic twins (genetically similar) but failed to find such a link in dizygotic twins (genetically different). This may suggest a genetic component to HMB [47].

The upregulation of COX enzymes expression and prostaglandin receptors enhances the production of vasodilatatory factors. This in turn leads to a downregulation in the expression of anti-angiogenic factors such as thrombospondin, angiostatin and endostatin which are potent vasoconstrictors. The increase in these vasodilatatory factors may further enhance menstrual bleeding and vascular dysfunction by further upregulating the COX–PG biosynthetic pathway via a positive feedback loop, and promote an autocrine–paracrine regulation of growth factors specific for vascular function such as VEGF [48, 49].

The increased expression of prostanoids in the endometrium of women with HMB has been targeted by cyclooxygenase enzyme inhibitors (COX-inhibitors) [50]. Prevention of PG synthesis is the mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs) such as mefenamic acid. Mefenamic acid works by reducing PG synthesis and suppressing PGE_2 binding to its receptors. This can reduce the number of days of bleeding and reduce menstrual blood loss by up to 35% [51].

All the above mechanisms play a role in altering the endometrial microenvironment in women suffering from HMB and most treatment methodologies have been aimed at correcting these.

1.9 Uterine Fibroids

1.9.1 The history of Uterine Fibroids

Uterine fibroids are benign tumours that develop predominantly in a woman's uterus. They also called myomas, leiomyomas, fibromyomas and fibroid tumours. However there has been a case of a fibroid developing in a male with a rudimentary uterus due to persistent müllerian duct syndrome [52]. The first mention of uterine fibroids was in Greek literature. Hippocrates (460-375 B.C) referred to them as uterine stones. Fibroids were later referred to as "scleromas" during the second century of the Christian period. Medieval texts refer to some women expelling eggs from their vagina in parturition which probably represented what is known today as fibroid expulsion. These women were accused of possessing evil spirits [53]. In 1854 Rudolph Virchow, a German pathologist, demonstrated that fibroids were composed from smooth muscle cells and later introduced the word myoma [54]. The modern term "fibroid" was introduced in 1860 by Karl von Rokitansky and in 1863 by his assistant Julius Klob.

1.9.2 The prevalence of uterine fibroids

Uterine fibroids are the most common benign tumours affecting women and are asymptomatic in 50% of women [55]. They may be single or multiple and their size varies from a maximum diameter of a few millimetres to up to 30 cm. They are highly prevalent and studies have shown that by the time women reach 50 years of age, nearly 70% of white women and more than 80% of black women will have had at least one fibroid although the majority will be asymptomatic [56]. Fibroids are the fifth leading cause of hospitalisations for gynaecologic conditions unrelated to pregnancy in women aged 15–44 years and the primary indication for hysterectomy among women of all ages in the United States. Around 30% of women presenting with heavy menstrual bleeding (HMB) will be found to have fibroids [57]. A recent study estimated annual direct costs (surgery, hospital admissions, outpatient visits, and medications) in the United States is over four billion dollars [58]. There is no similar UK wide cost estimation study, however, it is thought that surgical treatment of fibroids costs

the NHS around £62.5 million per year based on the 2012-2013 payments by results tariff [59].

Fibroids are a mixture of smooth muscle cells and fibroblasts, leading to hard, round, whorled tumours in the myometrium. The pathophysiology of fibroids remains unknown, although, it is hypothesised that each fibroid is derived from a mutation in a single smooth muscle cell [60]. It has been well established that oestrogen and progesterone are involved in the proliferation and maintenance of uterine fibroids and the majority of medical treatments currently available work by inhibiting sex steroids production or action [61]. It has also been shown that fibroids have a familial predisposition and that the frequency of newly diagnosed fibroids among first degree female relatives of women with fibroids is 2.2 times higher than in a non-familial group [62].

Case-control analysis has shown that the risk of developing fibroids was reduced with increased number of pregnancies and increased duration of oral contraceptive use. This suggests that unopposed oestrogen plays a central role in fibroid development and stimulation of growth [63]. The risk factors for developing fibroids are summarised in table 1-1.

Table 1-1: Factors that affect the risk of developing uterine fibroids

a-Age: Incidence increases with age during reproductive years [64]
b-Race: Incidence is higher in Black and Latina women than in white women[56]
c-Heredity: Risk is higher in women with first degree relatives who have fibroids [60]
d-Early menarche (before age of 11) [65]
e-Pregnancy: Full term pregnancy is related to lower rates of fibroids [66] also parous women experience lower rates of fibroids compared to nulliparous [57]
f-Hormonal contraception: OCP and progestin-only injectable contraceptives offers a protective effect against development of fibroids [57, 67]
g-Hormonal replacement therapy (HRT): while menopause reduces the risk of developing fibroids, the use of HRT increases it [67]
h-Obesity: Weight gain, central distribution of body fat are risk factors for fibroids [68]

1.9.3 **Fibroid symptoms**

The location of a uterine fibroid may impact on symptoms and quality of life but has no association with the size. Fibroids can cause menstrual problems, infertility or pressure symptoms which are usually associated with large fibroids compressing adjacent structures. When these symptoms impact on the quality of life of women, they usually require treatment. The symptoms women with uterine fibroids present with are summarised in table 1-2. [69]

Table 1-2: Symptoms which may be associated with uterine fibroids

1-Heavy menstrual bleeding (main symptom)
2-Sub-fertility/infertility
3-Dyspareunia
4-Pressure symptoms:
a-Pelvic pressure or discomfort
b-Abdominal distension/distortion
c-Urinary tract problems such as frequency, urgency, urinary incontinence and hydronephrosis
d-Lumbo-sacral spine: back pain & pelvic pain
5-Symptoms in pregnancy: miscarriage, pain, bleeding & placental abruption [70]
6-Severe pelvic pain secondary to fibroid torsion or ischemia secondary to fibroid degeneration [71]

1.9.4 **Fibroid location**

Fibroids can present in different locations. (Fig 1-4)
a-Intramural fibroids that grow within uterine muscle.
b-Submucosal fibroids that bulge into the uterine cavity.
c-Subserosal fibroids that project to the outside of the uterus.
d- Pedunculated that hang from a stalk inside or outside the uterus
e-Cervical (less common)

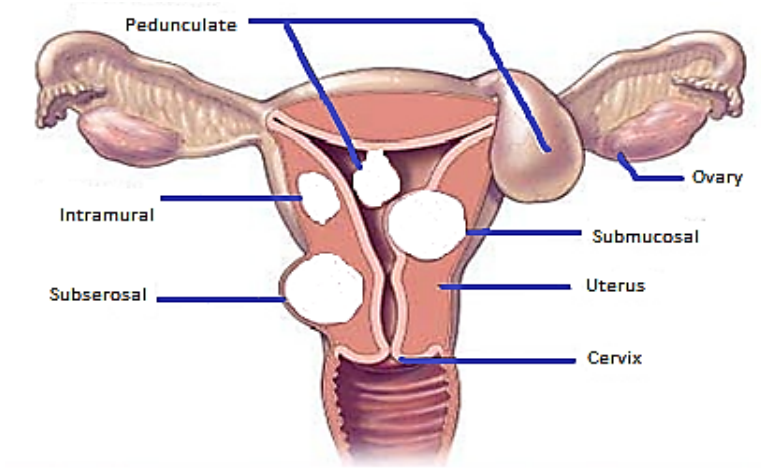


Figure 1-4: Fibroid locations

1.9.5 **Fibroids and menstrual disorders**

Heavy Menstrual Bleeding (HMB) is one of the main symptoms that fibroids can cause which has a major impact on a woman's quality of life.
The location of the fibroid in the uterus may have an impact on the clinical manifestations, for example submucosal fibroids seem to be associated with higher risk of developing anaemia secondary to HMB [72]. Although *Sulaiman et al* reported menstrual blood loss of more than 80ml in all women who had submucosal fibroids. He admitted in his study that menstrual blood loss does not correlate to the fibroid location [73]. Another study found that only 25% of patients with submucosal fibroids have HMB. Regardless of the location, uterine fibroids may have paracrine molecular effects on the adjacent endometrium that are extensive enough to cause HMB [74].

The exact mechanism by which fibroids cause HMB is still unknown and several theories have tried to explain this. One theory was that HMB associated with fibroids was a result of ovarian hormone imbalance [75]. However, this was refuted as no ovarian sex steroid difference has been found between women with fibroids and those without [76].

Increase in the endometrial surface area secondary to the presence of fibroids may explain HMB in some instances. *Chimbira et al* demonstrated that as the

uterus enlarges the surface area of the uterine cavity increases proportionately [77]. This larger surface area may also reduce the efficiency of uterine contractibility. Compression of fibroids on the venous plexus of the adjacent tissue leads to venous congestion of the myometrium and the endometrium which may lead to abnormal menstrual bleeding or HMB [69].

Fibroid tissue and normal myometrium are different. There is an increase in some matrix metalloproteinase, VEGF, transforming growth factor-b (TGF- ß) as well as plasminogen activators and inhibitors in fibroid tissue.
TGF- ß from fibroids adjacent to the endometrium may affect the endometrial stromal cells and lead to reduced production of plasminogen activator inhibitor (PAI), antithrombin III and thrombomodulin, which play a role in the local endometrial haemostasis [78, 79].

Dysregulation of angiogenic and regulatory growth factors may further contribute to heavy menstrual bleeding in women with uterine fibroids.
One of the causes of abnormal uterine bleeding may be the over-expression of growth factors or their receptors compared to that of the adjacent normal endometrium [79].

It has also been demonstrated that VEGF undergoes differential expression in the three components of human endometrium at various phases throughout the normal menstrual cycle. This suggests that VEGF has a functional role in the cyclical changes and remodelling process of the endometrium and any treatment for HMB in fibroids should theoretically alter the expression of VEGF [80].

1.9.6 **Fibroids and fertility**

It is difficult to say whether fibroids cause infertility and whether treating them improves it. Each should be answered individually as it also depends on other associated conditions that might impact on the chance of conception. The widespread use of ultrasonography has resulted in an increase number of women who present with sub-fertility being diagnosed with uterine fibroids. However, this may only be a coincidental finding rather than the main cause of infertility.

The role of fibroids in sub-fertility has been controversial for many years since the evidence is not strong and whether they should be removed, is still uncertain [81]. Due to the fact that fibroids are common in women of reproductive age, it is of no surprise that women presenting with sub-fertility, who are often older, have them. Fibroids may exert a negative effect on fertility even if the cavity is normal. This may be due to effects on uterine blood flow, impaired embryo implantation or abnormal sperm migration [82]. Despite this, many women with relatively large fibroids still achieve pregnancy and some experience no difficulty in becoming pregnant.

There have been studies that looked at the impact of fibroids on the success of in- vitro fertilisation (IVF) [83]. These studies often do not take into account that the mean age of women with fibroids who conceive is higher than the general pregnant population and the incidence of all complications increases with age.

Fibroids have the potential to cause a number of problems in pregnancy, including miscarriage. A systematic review of 23 studies concluded that spontaneous miscarriage rates were significantly higher in women with fibroids. However, no significant differences in preterm delivery rates were observed. This review reported improved ongoing pregnancy and live birth rates from 16 observational studies of myomectomy for intramural fibroids, but not from the four observational studies of myomectomy for submucosal fibroids [83]. It has also been shown that fibroids in close proximity to the placenta are more likely to be associated with bleeding in early pregnancy and spontaneous miscarriage [84]. This irregular bleeding seems to be similar to non-pregnant women with submucous fibroids [85].

The appropriate management for women who have fibroids and wish to retain their fertility or are actively trying to conceive is under review and further information in this area is very much needed. It is clear that subfertility resulting from fibroids is not absolute and many patients will conceive without intervention.

1.9.7 Diagnosing fibroids

1.9.7.1 Clinically

Women may present to their GP with one of the symptoms listed in table 1-2. The most common presentation is HMB or pressure symptoms. However, fibroids may also be an incidental finding when abdominal examination is performed for another reason. The impact on quality of life and other factors that may determine treatment options should be explored.
A clinical diagnosis of a fibroid uterus can often be made by an abdominal and pelvic examination. Findings of a firm, enlarged, and irregular shaped pelvic mass are characteristic of uterine fibroids although it can sometimes be difficult to distinguish a fibroids from an ovarian cyst or a malignant pelvic mass on clinical examination alone. Very large fibroids can be palpated abdominally as a solid mass extending from the uterus.
If a woman's symptoms are thought to be due to fibroids, an early referral to secondary care is advised as many of the treatment options offered in General Practice (GP) are unlikely to be successful in the presence of fibroids.

1.9.7.2 Ultrasound:

Pelvic ultrasound (trans-abdominally and trans-vaginally) is the most common initial investigation of a pelvic mass and can often be organised by the GP. It is a non-invasive, cheap, rapid and accurate investigation. Following referral to secondary care, a hysteroscopy may be carried out to assess the uterine cavity and whether any fibroids impinge on it. In a small study of 71 patients comparing trans-vaginal ultrasound versus hysteroscopy in diagnosing uterine fibroids, this showed ultrasound to have a sensitivity of 100% and specificity of 94% to predict the presence of fibroids in the uterus [86]. However its main limitation is the inability to distinguish between sub-mucosal fibroids and endometrial polyps necessitating a relatively invasive hysteroscopy to reach the diagnosis (fig 1-5). Transvaginal ultrasonography and outpatient hysteroscopy should be considered complimentary investigations and not alternatives when planning operative hysteroscopy [87].

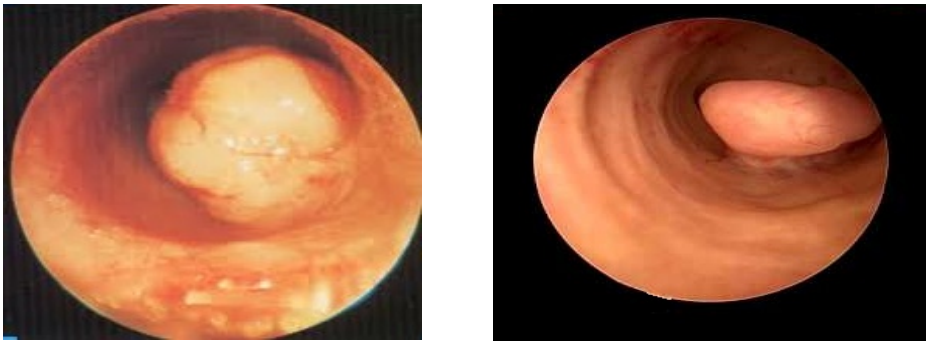


Figure 1-5: Fibroid visualised on hysteroscopy (left) and polyp visualised on hysteroscopy (right)

1.9.7.3 Magnetic Resonance Imaging (MRI):

MRI is increasingly being performed for the pre-intervention evaluation of uterine fibroids and the reasons for organising this are listed in table 1-3. The higher soft-tissue resolution of MRI compared with sonography allows more accurate determination of uterine and adnexal anatomy and might offer additional benefits over ultrasound. It should be considered in all patients prior to uterine artery embolisation as it assesses vascularity before and after treatment by measuring the contrast uptake of individual fibroids (fig 1-6). Further studies into myometrial and endometrial perfusion after uterine sparing surgery are needed to assess the specific effects on fertility and ovarian function this may well be by using dynamic contrast enhanced MRI (DCE-MRI) [26,27]. Diffusion weighted imaging (DWI) is a well-established MRI sequence that is used in cerebral imaging and its application to other parts of the body is relatively new. It is now being used as a part of the imaging protocols for pelvic malignancies and fibroids. In this thesis we will assess if using DWI offered any additional information over a standard MRI pelvis when imaging uterine fibroids.

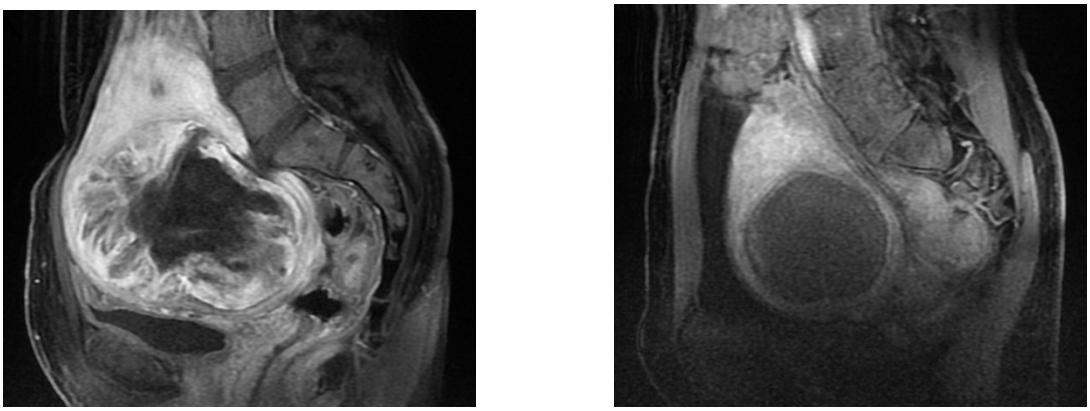


Figure 1-6: MRI of uterine fibroid showing contrast enhancement pre-UAE (left) and no contrast enhancement post-UAE (right)

Table 1-3: Advantages of pelvic MRI over pelvic ultrasound

1-Better soft tissue resolution
2-Easier to diagnose adenomyosis
3-Easier to visualise ovaries
4-More accurate measurements of fibroids and uterine volume
5-Assess suitability for uterine artery embolisation

1.9.7.4 Computerised tomography (CT):

CT scan is not the routine investigation of choice for the characterization of uterine fibroids. They are often found incidentally on CT scans that are requested for other various reasons such as those performed as part of a chest/abdomen and pelvis screening scan. Fibroids that are degenerating appear complex and contain areas of fluid attenuation. Calcification is seen in approximately 4% of fibroids and is typically dense and amorphous [88]. However, calcification can also be confined to the periphery of the fibroid when it is thought to be secondary to thrombosed veins from previous red degeneration. On contrast-enhanced scans, fibroids usually show low attenuation relative to the myometrium although, occasionally, they may be of the same or of higher attenuation. If the fibroid has undergone acute torsion, there may be enhancement of the rim of the fibroid due to obstructed peripheral veins but there will be no enhancement centrally. Fibroids can occasionally grow to massive sizes and present with symptoms secondary to mass effect, such as hydronephrosis.

1.9.7.5 Fibroids and malignancy: Leiomyosarcomas (LMS):

Fibroids are benign tumours while leiomyosarcomas are rare malignancies that may be difficult to distinguish clinically from fibroids. They can only be reliably diagnosed by histopathology. They can cause a great deal of concern since many women with fibroids either have no treatment or conservative options that preserve the uterus. Such treatment modalities do not lead to removal of the fibroids and therefore no histopathological diagnosis can be made. The incidence of leiomyosarcomas is low and it was thought that they represent only 1% of all uterine malignancies [89].
Leiomyomas (fibroids) do not generally develop into leiomyosarcomas [90]. One study reported leiomyosarcomas in 0.5% of hysterectomies for fibroids [91]. However a more recent review of 64 prospective studies found this rate to be less at 0.12% [92]. They usually present in women aged between 47 and 56 years of age but more commonly in post-menopausal women. Their symptoms can overlap with fibroids making the diagnosis difficult. Rapidly growing and painful fibroids, fibroids growing after menopause or growing despite GnRH use should raise suspicion. In a study by *Parker et al* the total incidence of uterine sarcoma among women operated on for having rapidly growing fibroids was 0.27% [93].

Dynamic contrast enhanced MRI using Gadolinium increases the likelihood of diagnosing leiomyosarcomas [94] and clinical applications of diffusion weighted MRI (discussed in chapter 5) may prove to be an effective and simple way of pre-operatively differentiating between benign and malignant disease of the uterus and hence early diagnosis of leiomyosarcomas [95].

1.9.8 **Treatment of fibroids:**

A historical perspective:

Ephraim McDowell in 1809 in Danville, USA performed the first laparotomy to treat a uterine fibroid. This was performed on Abraham Lincoln's 56 year-old cousin. Although the initial surgery was performed to relieve abdominal distension thought to be secondary to constipation, an ovarian cyst was removed which on later analysis was found to be a pedunculated fibroid [96]. The first successful myomectomy was performed in 1840 by Jean Zuléma Amussat in Paris. Myomectomy as a procedure was later abandoned due to the high mortality rate secondary to blood loss. It was later reintroduced in 1922 when Victor Bonney invented the myomectomy clamp which significantly reduced blood loss. He reported 403 myomectomy cases with low mortality rate [97].

Although hysterectomy is still widely offered as a treatment for uterine fibroids, there are different treatment options that should be first explored in order that women are able to give informed consent.
A combination of presenting clinical symptoms, fibroid size and location and the patient's desire to retain fertility all influence the choice of the therapeutic modality that can be offered and will be accepted by a woman today [98].

1.9.9 Nonsurgical Treatment of uterine fibroids

These treatments are aimed at symptom relief by reducing HMB and do not cure fibroids.

1.9.9.1 Tranexamic acid (Tx acid)

Tranexamic acid is a synthetic anti-fibrinolytic agent that is well tolerated orally. It provides a non-hormonal treatment for patients with excessive haemorrhage during the menstrual period [99]. It is used in many clinical situations in which the inhibition of fibrinolysis has shown to be of benefit in managing excessive bleeding. It is still unclear what the mechanism is for using Tx acid in treating HMB secondary to fibroids.
The anti-fibrinolytic effect can also cause complications such as thrombosis and embolism but the majority of studies haven't reported such adverse effects and tend to limit its reported side effects to mild headaches, allergy and discomfort [100].
A systemic review of the current evidence has shown that oral Tx acid may reduce menorrhagia in patients with fibroids. However, the review did not stratify fibroids by size or location [101].
The safety and efficacy of the modified release variant has been proven in women with fibroids when compared to placebo [99]. When given intravenously it has a role in decreasing peri-operative blood loss at myomectomy [102]. It is also being studied for potential use in postpartum haemorrhage [103].

1.9.9.2 Mefenamic Acid

Mefenamic acid is a non-steroidal anti-inflammatory drug (NSAID) that is commonly used for dysmenorrhoea. It leads to a modest reduction in HMB in women without fibroids although it is less effective than tranexamic acid [104]. One small study indicated that Mefenamic acid reduced blood loss in women with anovulatory dysfunctional uterine bleeding and fibroids[41]. However, there have been no large trials to date to show the benefits of NSAIDs in women with fibroids.

1.9.9.3 Levonorgestrel-releasing intrauterine system (LNG-IUS)

Levonorgestrel is a second generation progestin which is used as an active ingredient in some hormonal contraceptives. Chemically it is a gonane progestin derived from 19-nortestosterone. It is a hormonally active levorotatory enantiomer of the racemic mixture norgestrel and hence its name levonorgestrel [105].

The LNG-IUS is marketed under the trade name MIRENA™. It was developed by Luukkainen and first released in 1976 by manufacturer Bayer AG and used for contraception [106]. It provided a safe and highly effective form of contraception for up to 5 years. Its main role was to mitigate the increased menstrual bleeding associated with copper and inert IUDs.

It has a range of non-contraceptive uses including treatment of heavy menstrual bleeding with/without fibroids, endometriosis, endometrial hyperplasia and can be used in combination with oestrogen for hormone replacement therapy in non-hysterectomised women to avoid endometrial hyperplasia.

Levonorgestrel-releasing intrauterine system consists of a T-shaped polyethylene frame with a steroid reservoir around the vertical stem. The reservoir consists of a white or nearly white cylinder, made of levonorgestrel, containing a total of 52 mg levonorgestrel. The reservoir is covered by a semi-opaque silicone (polydimethylsiloxane) membrane. The T-body is 32 mm in both the horizontal and vertical directions. The polyethylene of the T-body is compounded with barium sulfate, which makes it radiopaque. A monofilament brown polyethylene removal thread is attached to a loop at the end of the vertical stem of the T-body.

Following intrauterine insertion of the LNG-IUS, the initial release rate of levonorgestrel is 20μgm per day. This provides a stable plasma levonorgestrel concentration which, after the first few weeks, stabilizes between 150 to 200 pg/mL in women of fertile age. It releases levonorgestrel locally into the endometrium, causing endometrial atrophy by suppressing endometrium proliferation, while minimising systemic doses and side-effects. The thin atrophic endometrium is characterised by fragile blood vessels and are sub-optimal for implantation. An ultrasound scan to rule out significant uterine

cavity distortion is usually carried out prior to insertion. A follow up 6 weeks after insertion is carried out to check for expulsion. The overall incidence of spontaneous LNG-IUS expulsion is 9.6% over a three year period. This is increased to 15.8% in the presence of fibroids [107].

The LNG-IUS itself does not affect the size of the fibroids and doesn't lead to shrinkage. A prospective, comparative study of the efficacy of LNG-IUS in the presence of fibroids demonstrated a marked reduction in mean blood loss (MBL) in both women who had idiopathic HMB and those who had HMB related to small fibroids less than 50ml in volume. However, there was no significant reduction in fibroid volume after a 2 year follow-up period compared to baseline [108]. This was echoed by another prospective cohort study comparing HMB and uterine volume in women with uterine fibroids using LNG-IUS and a control group using LNG-IUS for contraception. This showed that LNG-IUS significantly reduced uterine volume in both groups but there was no significant reduction in the volume of the fibroids themselves [109].

1.9.9.4 Gonadotrophin-releasing hormone (GnRH) agonists

Gonadotropin-releasing hormone (GnRH), also known as luteinising-hormone releasing hormone (LHRH) is a trophic peptide hormone responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland (fig 1-6). GnRH is synthesised and released from GnRH neurons within the pre-optic anterior hypothalamus. It is the target of various regulatory mechanisms of the hypothalamic-pituitary-gonadal axis and is inhibited by increased oestrogen levels in the body. The pulsatile secretion pattern of GnRH affects the synthesis and release of LH and FSH. A high GnRH pulse frequency is more effective in releasing LH than FSH [110]. However, low pulse frequency supports FSH synthesis and release but is not as effective in increasing LH concentration. On the other hand, high GnRH pulse frequency inhibits FSH synthesis and release [111] .

GnRH agonists are derived from native GnRH by substitution of a D-amino acid for the native L-amino acid at position 6 in the decapeptide. This substitution

yields the agonist resistant to degradation and increases its half-life and the time of receptor occupancy [112].

A constant intravenous infusion of GnRH or administration (subcutaneous/ intramuscular/ intranasal) of GnRH agonists causes an initial agonistic action known as the flare phenomenon [113]. This is then followed by down-regulation of receptor concentrations, which desensitizes the pituitary to continued stimulation. The flare phenomenon is caused by the release of the gonadotropins which are already produced and stored in the pituitary. It is greatest in the early follicular phase when GnRH and oestradiol have combined to create a large reserve pool of gonadotropins. Within 3 to 4 weeks, it induces a hypogonadotropic-hypogonadal anovulation state, a situation simulating WHO Group-1 anovulation [114]. This is referred to as “switching off” the ovaries temporarily and can be used for the treatment of uterine leiomyomas [115].

Randomised, controlled trials have demonstrated that the use of GnRH agonists for 3 to 4 months prior to fibroid surgery reduces both uterine & fibroid volume. However, this effect is only present during therapy and withdrawal of GnRH treatment results in fibroids returning to their original size within a short period of time [116].

GnRH agonists are beneficial in correcting pre-operative iron deficiency anaemia reducing intra-operative blood loss & operating time and decreasing hospital stay. When uterine size is such that a mid-line incision is planned, this can be avoided in many women with the use of GnRH agonists [117]. There are, however, doubts as to their benefits prior to myomectomy [118]. A Cochrane review found that they are not cost-effective [117]. One double-blind placebo controlled randomised trial did not support pre-operative GnRH agonist therapy for hysteroscopic resection of fibroids as no benefit in such treatment was identified [119]. Add-back therapy should be initiated at the same time as the treatment with a GnRH agonist if use for more than 6 months is anticipated. This reduces the hypo-estrogenic side effects such as vasomotor symptoms and loss of bone mineral density and mostly preserves agonist efficacy [120].

1.9.9.5 Selective progesterone receptor modulators (SPRM):

SPRMs are a relatively new class of synthetic ligands with tissue-selective effects of mixed agonist and antagonist activity. The first member of this class was mifepristone which has unique antagonist properties. Its use currently is predominantly in termination of pregnancy [121]. The anti-progesterone properties of mifepristone have also been utilised for the treatment of fibroids and have been shown to be effective in reducing fibroid size and improving the quality of life at low doses[122]. Research has shown that progesterone augments fibroid proliferation, raising the possibility that selective progesterone receptor modulators (SPRM) could inhibit fibroid growth [123]. This research has culminated in the emergence of ulipristal acetate (UPA) for the treatment of fibroid related symptoms.

Three randomised controlled trials on UPA have now been published using varying doses and duration of use HMB [124-126]. Comparison has been made with GnRH agonist and also of 2-three month courses with and without a progestagen. These showed that it had some preoperative benefit of shrinking fibroids sized between 3cm to 10cm and was very successful in relieving HMB. UPA induced amenorrhea in women with fibroids and controlled menstrual bleeding, however, irregular bleeding occurred in those with sub-mucous fibroids [85, 124].

Unlike GnRH agonists, UPA does not induce bone loss and climacteric side effects as endogenous oestrogen secretion is maintained [127]. The potential long term effects of UPA on the endometrium are under study. An unusual histological pattern, “non-physiologic” endometrial change has occurred in the majority of women treated with UPA. These changes are thought to be benign as there is no increase in proliferation and no increase in hyperplasia [128]. It should only be used for treating HMB, if it is thought to be caused by fibroids [125]. Common side effects for its use are headaches, nasopharyngitis and abdominal pain. The issue of endometrial effects of these compounds remains to be resolved, however, other promising applications, including long-term contraception, treatment for endometriosis, endometrial cancer, Cushing's disease and Alzheimer disease are currently in development [129].

There has been a recent publication of a case series of 18 pregnancies following UPA treatment of uterine fibroids. The initial results are promising with a 71% pregnancy rate and a subsequent two thirds of these resulting in a live baby [130]. Further research is needed before recommending UPA to all women with fibroids contemplating pregnancy.

1.9.9.6 Other Medical Treatments

Other medical treatments can be used although frequently, they are less effective in the presence of fibroids. Treatments that inhibit ovulation and/or decrease oestrogen levels such as the oral contraceptive pill, norethisterone acetate and depot medroxyprogesterone may be valuable. Aromatase inhibitors in premenopausal women and have been shown to reduce the size of uterine fibroids, improve symptoms and are generally well tolerated [131].

1.9.10 Radiological Treatments

1.9.10.1 Uterine Artery Embolisation (UAE)

UAE as a treatment for symptomatic uterine fibroids was first described in 1995 [132]. Prior to this it was used for massive obstetric haemorrhage. It is indicated for symptomatic fibroids and is an alternative to myomectomy since it allows conservation of the uterus and also only involves a short hospital stay. UAE is a minimally invasive treatment option for uterine fibroids performed by an appropriately trained interventional radiologist. A catheter is inserted through the femoral artery in the groin under local anaesthetic and directed towards the uterine arteries using fluoroscopy (Fig 1-7). The uterine artery is blocked on each side using an appropriate embolic agent. The objective of UAE is to completely infarct all the fibroid tissue while preserving the uterus, ovaries and surrounding pelvic structures. The most common problem associated with UAE is post procedure pain which can usually be controlled by opiates (appendix 4). Expulsion of a necrotic fibroid, chronic vaginal discharge and premature ovarian failure are less common effects of UAE.

A recently published Cochrane review summarized the results of five RCTs comparing UAE versus surgery (hysterectomy/myomectomy). Two of these studies included a 5-year follow-up. Satisfaction with treatment was the primary outcome measure and the pooled results showed no short-term or long-term differences in satisfaction rates between the UAE and the surgery group [133]. Major complications were rare and no reported difference was found between the two groups. However, there were more minor complications in the UAE group [133].

The evidence is still lacking regarding fertility after UAE. It has been derived from small studies since, up until recently, the intention to conceive was considered a contraindication to the procedure by some gynaecologists and radiologists. Patients were actively excluded from several UAE studies on that basis [134]. Pregnancies in women who have had UAE have been carefully followed up and some reports have suggested an increase in complications of pregnancy.

UAE is also associated with degradation of ovarian function which is mainly seen in women > 45 years old with little evidence of an impact in women < 40 years of age [135].

It is not fully understood how uterine artery embolisation works in women with HMB. Some women achieve a significant reduction in their fibroid size and uterine size but continue to suffer from HMB. Other women have minimal reduction in fibroid and uterine size but have good symptom relief.

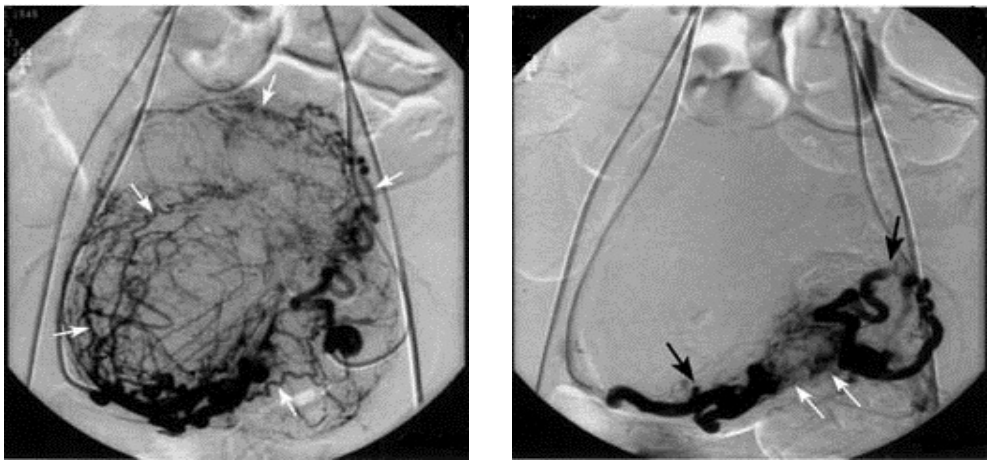


Figure 1-7 Fluoroscopy pre-UAE (left) showing the uterine artery coiling and post-UAE (right) showing stasis of contrast material and blood flow

1.9.10.2 Magnetic Resonance-Guided High Intensity Focused Ultrasound (MRgHIFUS)

MRgHIFUS is a relatively new technique that was first performed in 2002 [136] . It is a thermo-ablative technique that uses focused high-energy ultrasound (HiFU) to cause coagulative necrosis at a precise focal point within the body. The focal point is the point where ultrasound waves converge and the distance between the transducer and the focal point can be altered. The location and extent of treatment can be monitored accurately with real-time magnetic resonance imaging (MRI) and MRI thermal mapping [137, 138]. MRI provides a three-dimensional view of the targeted fibroid tissue allowing precise focusing and delivery of the ultrasound energy. Immediate post-procedure MRI of the treated area aids in determining success.

In the short term MRgHIFUS has proven to be a safe and effective non-invasive method of managing symptomatic fibroids [139]. The disadvantage is that the treatment time is long and relatively few patients are eligible, since only those with fibroids located immediately beneath the anterior abdominal wall and without scars in the region of interest are suitable. [140]
MRgHIFUS can be performed on an outpatient basis and is associated with short recovery time. In women with future childbearing plans and a single uterine fibroid, it can be an effective treatment alternative. Despite a high rate of intervention following treatment, MRgHIFUS benefits from being a non-invasive and uterine-sparing treatment modality [141]. Obstetric outcomes following MRgHIFUS are encouraging but further research is required especially to compare the outcome with more established procedures such as UAE and myomectomy [142].

1.9.10.3 Other ablation procedures: The Vizabalate and Sonata System

This system includes a high resolution, ultra-compact sonography probe at the tip of a radiofrequency ablation device. It is designed to be introduced into the uterine cavity similar to a hysteroscope. It can image and treat submucosal fibroids using a SMART (Setting Margins Ablation in Real Time) targeting system. This system is used to place the ablation probe in the centre of the fibroid and selectively apply heat cause fibroid coagulative necrosis.

This approach of using radiofrequency ablation under laparoscopic/hysteroscopic guidance to treat symptomatic fibroids was first introduced in 2002 [143]. This has led to the development of the study device Acessa™ which carries out laparoscopic ultrasound guided radiofrequency volumetric thermal ablation of uterine fibroids (RFVTA). It has the benefit of delivering high frequency ultrasound directly to the fibroid. RFVTA of uterine fibroids has resulted in sustained relief from fibroid symptoms and low repeat intervention rates [144].

1.9.11 Surgical Treatment

1.9.11.1 Hysterectomy

Hysterectomy is the established treatment of fibroids for women who no longer wish to retain their fertility. The first successful selected hysterectomy operation was performed in 1813 by Conrad Langenbeck via the vaginal approach [145]. Approximately 55,000 hysterectomies are performed each year in the UK and over 600,000 in the USA [146, 147]. Around one third of these hysterectomies are performed to treat uterine fibroids[147].

Although hysterectomy is a major surgical procedure, it results in significant resolution of symptoms and has a high satisfaction rate. The cost benefit of this procedure was accounted for in the REST trial, an RCT of UAE versus surgical treatment for fibroids. Although there was initial cost benefit of UAE over surgery at 12 months, this was substantially reduced, because of subsequent interventions, with UAE and surgery being cost neutral at 5 years. [148]

Hysterectomy is associated with procedure related morbidity and mortality and is often the non-preferred option to treating fibroids. Complications can include haemorrhage, infection, injury to bladder and/or urethra, bowel injury, blood transfusion, increased risk of future prolapse and anaesthetic risks.

Two patient preference studies showed that women with HMB secondary to fibroids and other causes tend to prefer alternative treatments that are only 50% successful in order to avoid a hysterectomy. Although many will have a hysterectomy at a later date [149]. Minimally invasive surgery rates for hysterectomy (both laparoscopic and robotic) have increased over time and patient experience has been shown to be better than that for open abdominal surgery [150]. Perhaps the future will see an even more significant move towards minimally invasive approaches for all gynaecology surgery both for benign and malignant causes.

1.9.11.2 Myomectomy:

Myomectomy is a fertility sparing option to surgically remove fibroids and preserve the uterus. However, it can be associated with life-threatening bleeding, risk of hysterectomy, prolonged postoperative stay, post-operative adhesion formation and recurrence [98]. Depending on the size and location of the fibroid it can be performed as an open procedure, laparoscopically, robotically or vaginally using hysteroscopy. Some of these procedures require specific skills that are often only available in specialist units. There is no difference in morbidity between patients undergoing abdominal myomectomy and those undergoing abdominal hysterectomy.
The data regarding fertility and pregnancy outcomes after myomectomy is incomplete and the effect of myomectomy on future pregnancies is understudied.

1.9.11.2.1 Abdominal Myomectomy (laparotomy)

Is defined as removal of a single or multiple uterine fibroids through an abdominal incision and conserving the uterus. Abdominal myomectomy was first reported in 1845 and was performed by Dr Washington Atlee Burpee in Pennsylvania [151]. Prior to this hysterectomy was the main treatment for women who presented with symptomatic uterine fibroids due to the associated bleeding thought to occur with myomectomy [152]. As blood transfusion was introduced and its safety vastly improved, more successful myomectomies care now being achieved.

It is perceived that women whom opt for myomectomy are doing so to retain fertility & improve chances or conception. A recent observational study has suggested that abdominal myomectomy might improve reproductive outcomes in patients with fibroids. The reproductive performance was especially good with younger patients and those that had previous pregnancies prior to myomectomy. It was also associated with lower miscarriage rate after pregnancy compared to those prior to surgery [153]. However, there is currently insufficient evidence from randomised controlled trials to evaluate the role of myomectomy to improve fertility [154].

1.9.11.2.2 Laparoscopic myomectomy

Laparoscopic myomectomy has long been the only minimally invasive treatment option for symptomatic fibroids, prior to the introduction of UAE. It can be used to remove subserosal and intramural fibroids depending on the size and position of the fibroid and the skill of the surgeon [155] .

The rate of conversion of laparoscopy to laparotomy and all major complications have been reported to be very low [156]. Pregnancy outcomes are encouraging and major complications such as uterine rupture are reported as rare [157]. Further studies into the strength of uterine scars following laparoscopic myomectomy in comparison with open myomectomy should be conducted [158].

It seems reasonable for surgeons to adhere to techniques developed for abdominal myomectomy including limited use of electrosurgery and multi-layered closure of the myometrium. Nevertheless, individual wound healing characteristics may predispose to uterine rupture [159]. Laparoscopic myomectomy, when performed by an experienced surgeon, can be considered a safe technique, with an extremely low failure rate and good pregnancy outcomes. The pregnancy rates for women with fibroids, managed by this method are similar to those after laparotomy [160].

1.9.11.2.3 Hysteroscopic Myomectomy

A hysteroscope is a form of gynaecological endoscope that carries optical and light channels. It is introduced into the uterine cavity covered by a protective sheath through the cervix. The sheath provides an inflow and outflow channel for insufflating the uterine cavity with either gas or fluid.
Submucosal fibroids can often be removed by hysteroscopic myomectomy. This can be done under general anaesthetic or local anaesthetic as an outpatient procedure under the auspices of office gynaecology. It is however dependant on the size of the fibroid. Despite the well-known effect of reduction in bleeding after removing a submucosal fibroid, there is little evidence from randomised studies to support this [155].

The most common perioperative complications associated with hysteroscopic myomectomy are haemorrhage, uterine perforation, cervical laceration and fluid overload [155]. Intrauterine adhesions (Asherman's syndrome) can be a delayed complication and has a reported incidence of 10% at second look hysteroscopy. It seems to be higher when resecting multiple submucosal fibroids and those that have an intramural element [161]. Such adhesions can be treated successfully in an outpatient hysteroscopy setting [162].

Hysteroscopic myomectomy of submucosal fibroids seems to increase the odds of clinical pregnancy in women with unexplained subfertility. It also appears to increase pregnancy rates and decrease miscarriage rates, however, the evidence at present is still inconclusive [163].

1.9.11.2.4 Robotic surgery

The Food and Drug Administration (FDA) approved the Da Vinci surgical system in 2005 for gynaecological surgery. The Stanford Research Institute along with the defence department developed the Da Vinci system initially so that surgeons sitting remotely from the battlefield could perform tele-surgery on wounded soldiers. It comprises of three components (Fig 1-13):
1-A surgeon's console
2-A high-definition three-dimensional (3D) vision system
3-A patient-side cart with robotic arms

The first component is the surgeon console where the surgeon sits away from the patient and uses a stereoscopic viewer with hand manipulators and foot pedals that allow control of the robot-assisted instruments within the patient. The second component is the vision system, which provides the 3-dimensional image through a 12-mm endoscope containing stereoscopic cameras and dual optical lenses. The third component is the patient-side cart with telerobotic arms and endowrist instruments. One of the arms holds the laparoscope while the other 2 to 3 arms hold the various laparoscopic surgical instruments. The endowrist instruments are unique in that they possess a mechanical wrist that replicates

the full range of motion of the surgeon's hand as controlled from the surgeon console. The advantages of this robotic technique are smaller incisions, leading to lower morbidity, less postoperative pain and shorter hospital stays which are similar to any minimally invasive surgery. However, robotics do seem to have an edge in highly complicated procedures when extensive dissection and normal anatomy re-establishment is required [164].

One of the first series of myomectomy using the Da Vinci robot included 35 patients and was reported in the literature by Advincula *et al*. [165]. The conversion rate from robotic to laparotomy was comparable to that of conventional laparoscopic myomectomy. Robotic surgery has also shown less intraoperative blood loss, shorter hospital stay and fewer complications when compared to abdominal myomectomy. Operative time however is longer when compared with conventional laparoscopies [166].

Pregnancy outcomes following robotic assisted myomectomies have been studied and were found to be similar to open surgery [167]. This new promising field is undergoing significant research and development at present.

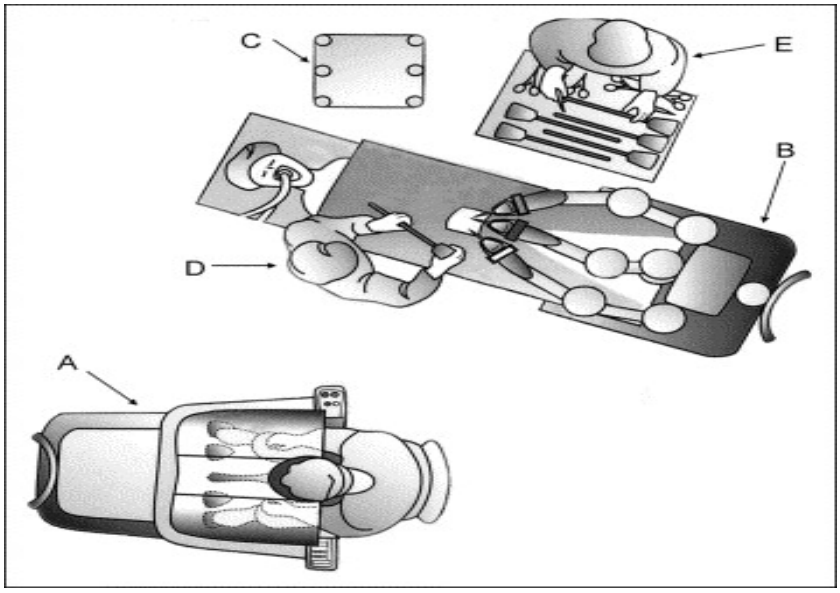


Figure 1-8 Robotic surgery layout showing centre console and patient

A: Console and first surgeon, B: Robot, C: Open surgery tray, D: Second surgeon & E: Scrub nurse

1.10 Aim and hypothesis

Uterine artery embolisation (UAE) is a unique procedure used to treat HMB associated with fibroids but its mechanism of action is unknown. The usual result of this procedure is a necrotic fibroid that is smaller than pre-UAE mass and symptom resolution. The blood supply to the fibroid is completely occluded and this is evident by lack of contrast enhancement in an affected fibroid despite the rest of the uterus appearing to be unaffected. Taking a biopsy from the endometrium is usually tolerated well by women. By obtaining such biopsies both before and after UAE means that differences in the expression of various endometrial markers can be studied. Women undergoing UAE usually have an MRI before and after treatment. The high quality imaging of the fibroid uterus with MRI allows an objective and reproducible comparison of the different components of a fibroid uterus before and after applying treatment this treatment method. This thesis is based on the hypothesis that uterine artery embolisation alters the expression of certain angiogenic factors such as VEGF and down regulates the COX-PG biosynthetic pathway leading to improvement of heavy menstrual bleeding. Despite this change, the function of endometrium is not affected and no long term damaging to the uterus is visible.

The two main aims are:

1-To study the effect of UAE on the endometrium of women with HMB. This was done through looking at the changes in the expression of VEGF-A and COX-2 and also through assessing the changes in microvascular density (MVD) and endometrial proliferation after UEA.

2-To look at the morphological changes occurring in the fibroid uterus after UAE and whether these changes are governed by the size and location of the dominant fibroid. We also looked at other factors that may influence the outcome after UAE such as age of the patient at the time of UAE, the size of the uterus and whether a single fibroid uterus responds differently to UAE than a multi-fibroid uterus. We will explore the success rate by blood loss measurement and fibroid shrinkage and the general complications that occur.

Chapter 2

2. Methods and materials

2.1 Subjects

The study subjects in this thesis were pre-menopausal women who had heavy menstrual bleeding secondary to uterine fibroids. These women underwent uterine artery embolisation (UAE) as a treatment modality to relieve symptoms associated with uterine fibroid.

Study 1 (Prospective histological study)

Endometrial biopsy specimens were collected from women that were booked to undergo uterine artery embolisation. Each study participant presented with a fibroid uterus containing variable numbers, sizes and locations of fibroids (Fig 2-1).

All these women presented mainly with the complaint of heavy menstrual bleeding (HMB). Uterine fibroids in all participants were diagnosed initially at a gynaecology outpatient clinic by pelvic ultrasound after being referred by a general practitioner (GP). They were then referred to the tertiary interventional radiology clinic at Gartnavel Hospital in Glasgow. The different options of treating fibroids were discussed again with each patient & written information was provided. Each patient then underwent a contrast enhanced MRI prior to uterine artery embolisation to further evaluate the number, location and size of the fibroids. The contrast was used to assess fibroid tissue enhancement thus reflecting fibroid viability and suitability for UAE.

Although 23 patients were initially recruited to participate in this study, four patients did not subsequently have a UAE procedure. One of the patients was found to have an incidental right ovarian teratoma which required a laparotomy. Three patients changed their mind and requested a hysterectomy instead. Endometrial sampling was attempted both before and after UAE in 18 of the remainder 19 patients as one patient could not tolerate endometrial sampling and was therefore excluded from the study.

Although 36 samples (18 before and 18 after) were obtained, only 26 samples from 13 different patients included endometrial tissue that was suitable for

dating and testing. The samples that were not analysed included those with chronic endometritis, cervical tissue samples and those that were deemed insufficient samples for dating.

The study protocol had been previously approved by the ethics committee West of Scotland REC 5, REC reference number (10/S1001/1) and favorable ethical opinion was given on 04/05/2010. A substantial amendment was submitted to allow access to the Diffusion weighted MRI images and reports of all the patients in this study and to include them in this research. A favorable opinion of the amendment was given on 18/03/2015. Participants filled in a questionnaire obtaining their demographic and clinical data. All women signed a consent form agreeing to participate in the study & to give permission to access their data and inform their GP (Appendix 1). They were asked to complete the Pictorial Blood loss Assessment Chart (PBAC) for one period prior to UAE and one period 6 months after UAE. The subjects' details will be addressed in the results section of each chapter. (Appendix 1)

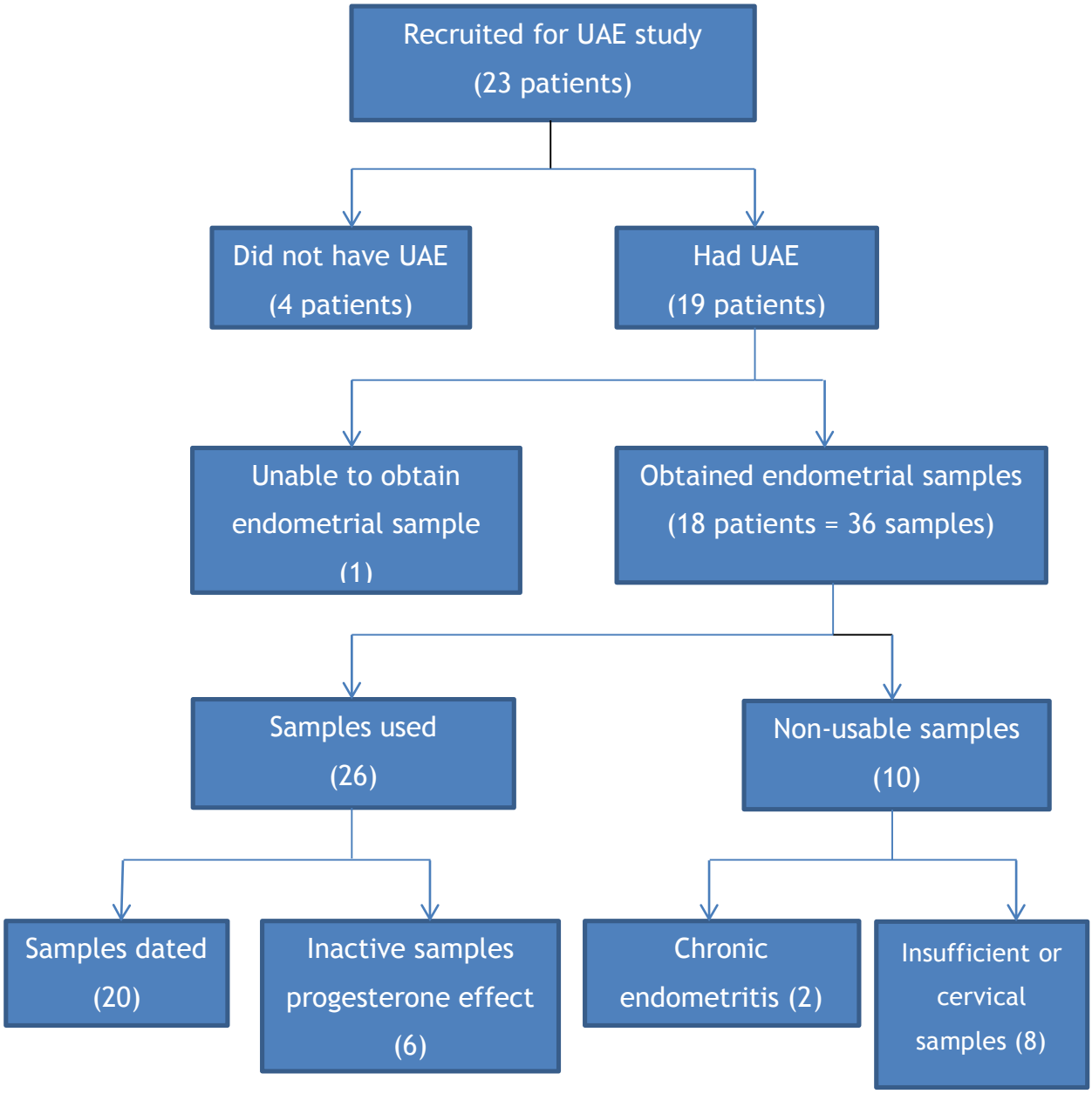


Figure 2-1 Flow chart of patients recruited for the endometrial biopsy study

Table 2-1: Summary of demographic & endometrial sample details of participants in study 1

The character	**Pre-UAE group (N= 13)**
Age (mean ± STD)*	47.1 ± 3.6
BMI (mean ± STD)**	32.1 ± 6.4
Parity (Number of children)	
Nil	N=4
1	N=3
2	N=5
3	N=1
Length of period pre-UAE:	
5-7days	N=5
7-10 Days	N=5
>10 days	N=3
Number of uterine fibroids in the uterus:	
1 fibroid	N=2
Multi-fibroids	N=11
Location of dominant fibroid:	
Unable to identify	N=2
Submucosal	N=2
Subserosal	N=1
Intramural	N=8
Stage of endometrial samples obtained:	
Inactive	N=6
menstrual	N=0
Proliferative	N=10
Secretory	N=10

*Age range (37-50)
**BMI range (22.1 – 40.8)

Study 2 (Retrospective MRI study)

We collected & analysed five years of MRI image data from patients who had undergone UAE between 1st of January 2010 and 31st of December 2014 in NHS Greater Glasgow and Clyde. It was not possible to retrospectively look at data prior to 2010 as the patient's electronic record system had not been introduced to NHS GG&C prior to this.
The end date for this period (31st December 2014) ensured that data from 18 months follow up post-UAE would be available for analysis to include in this thesis.
The study population was identified using the Clinical Research Information System (CRIS) at Gartnavel Hospital, Glasgow using a search by exam name: Embolisation of uterine fibroid, code IUTFDE. Follow up post UAE and re-admission rates was identified through the Information Services Division (ISD) Scotland. Exclusion criteria were applied and this will be discussed further in chapter 4. The total number of patients in this group n=133 who had MRI images available pre-UAE & 6 months post-UAE.

Caldecott guardian approval was obtained and the local R&D team at the Western Infirmary, Glasgow approved the study and individual consent was waivered. Using the Research Application System (IRAS) the Proportionate Review ethics Sub-Committee reviewed the ethics application and gave a favourable opinion (Appendix 3).

Measurements of the uterus and the dominant fibroids were undertaken on the MRI images pre & 6 months post-UAE. The volumes were then measured and compared. The number of fibroids was counted in each patient and the location of the dominant fibroid identified. Further details will be discussed later in this chapter (Section 2.5)

Table 2-2: Summary of Age distribution and fibroid data for study 2

Character	Group of patients N= 133
Age (Mean ± STD) <35 35-40 41-45 46-50 >50	44.3 ± 6.1 N=10 N=20 N=40 N=43 N=21
Pre-UAE Volume of: Uterus (Mean ± STD) Dominant fibroid (Mean ± STD)	 = 882.28 ± 660.9 cm^3 =385 ± 407.2 cm^3
Number of fibroids in the uterus: Unable to distinguish One Two Multiple	 N=3 N=40 N=7 N=84
Location of dominant fibroid: Unable to identify Submucosal Subserosal Intramural Intramural and part submucosal	 N=5 N=9 N=41 N=69 N=9

2.2 Histological study

2.2.1 Collection of biopsy specimens

2.2.1.1 Uterine biopsy specimen collection

The Pipelle® endometrium sampler (Williams Medical) was used to obtain endometrial biopsies from women who underwent uterine artery embolisation on two separate occasions.
The first endometrial biopsy was taken prior to UAE and a mutually convenient appointment was made for the woman to attend the hysteroscopy outpatient clinic in Stobhill hospital. However, some women expressed preference to have the endometrial sample taken at the time of the UAE procedure and this was arranged in ward 56A at Glasgow Royal Infirmary. The second endometrial biopsy was taken 6 months after the UAE procedure at the hysteroscopy outpatient clinic in Stobhill Hospital. We timed the second biopsy to be taken at the same stage of the menstrual cycle as the first sample relying on the patients last menstrual period date.

2.2.1.2 Blood samples specimen collection

Two 5ml blood samples were obtained in a lithium heparin yellow bottle at the time of collecting endometrial samples. One sample was processed by biochemistry laboratory at Glasgow Royal Infirmary. This was used to obtain luteinizing hormone (LH), follicular stimulating hormone (FSH), oestradiol & progesterone levels. The second sample was stored for future research.

2.2.2 Handling and storage of biopsy specimens

2.2.2.1 Handling and storage of endometrial biopsies

The presumed endometrial biopsy that was obtained was divided immediately into two parts. The first part was inserted into a histological specimen collection pot that contained 4% formaldehyde. This was sent to the central pathology department at the Queen Elizabeth University Hospital (formerly the Southern General Hospital) in Glasgow were it was processed and embedded in paraffin and was reported by a pathologist. The second part was placed directly into RNA later solution, Ambion R 0901 RNA Later® (0.5-1 cm of tissue sample in 5-10 ml of the solution respectively) and stored in -20C° freezer (Reproductive and Maternal Medicine laboratory, McGregor building, Glasgow Western Infirmary Hospital). After two weeks the biopsies were transferred to a cryo-pot by using liquid nitrogen and stored in -80 C° until used. After the transfer of the university laboratory from the McGregor building to the New Lister Building, liquid nitrogen was not immediately available. Therefore the process of transferring the biopsy samples from RNA later to a cryo-pot was changed to accommodate this. The cryo-pot was cooled down by leaving in a -80 freezer overnight. It was taken out of the -80 freezer and sat on dry ice just prior to transferring the biopsy sample from RNA later into it. This ensured that the sample would freeze immediately however it is a deviation from the original method using liquid nitrogen. Liquid nitrogen became available again later in the study.

On certain occasions it was not possible to split the Pipelle® sample into two separate samples as there was minimal tissue. On such occasions the whole tissue specimen was sent to the histopathology laboratory and was processed into a paraffin block process. RNA was then extracted from the blocks (Section 3.5.5).

2.2.2.2 Handling and storage of blood samples

Blood samples were centrifuged at 13000 rpm for 15minutes at 4C°. The serum was split into 4 tubes (0.5ml in each tube) and stored in a -80C° freezer. 1 ml serum from each sample was sent to the biochemistry department at Glasgow

Royal Infirmary where oestradiol, progesterone, luteinizing hormone (LH) and follicular stimulating hormone (FSH) assays was carried out using ARCHITECT reagent for each assay through the ARCHITECT í System (Section 3.5).

2.2.3 **Blood loss assessment**

We used the Pictorial Blood Loss Assessment Chart (PBAC) to estimate vaginal blood loss during a period. This is validated chart and is internationally used [168].
Participants who were awaiting uterine artery embolisation and agreed to join the study were sent the PBAC form with instructions on how to use it. They were asked to fill the form for one menstrual period prior to the procedure. Participants indicated the degree to which the sanitary wear was soiled by indicating the number of slightly, moderately and heavily soiled pads and tampons that they used. This was then repeated for one other menstrual period, 6 months after the UAE procedure. Assessment of blood loss using this chart and the results of comparing pre & post-UAE results are discussed in chapter 6.

2.2.4 **Histological dating**

Histological dating was carried out according to *Noyes et. al.* criteria to confirm the stage of the menstrual cycle by Professor Alistair Williams, University of Edinburgh (fig 3-9) [169]. All histological stages were consistent with the patients' last menstrual period (LMP) and the circulating oestradiol and progesterone concentrations in venous blood obtained at the time of tissue collection. Consistency between these parameters has been approved to be a robust method for characterising the stage of the cycle in endometrial samples [170]. Some endometrial samples were reported as inactive secondary to an exogenous progesterone effect and we will discuss this further in chapter 3. Another group of samples were deemed unsuitable for dating. These included samples that did not contain endometrial tissue, those that only had scanty tissue and those with chronic endometritis. Chronic endometritis samples were excluded as there has not been any human study looking at the effect this has on the markers we studied. There has however, been one study on gilts endometrium that looked at COX-2 expression in acute & chronic endometritis

compared to normal endometrium. This suggested COX-2 expression might be dependent on the duration of exposure to inflammatory agents [171]. As such duration was not known in our study nor is there a known standard range for COX-2 expression, we excluded these samples.

2.3 Immunohistochemistry (IHC)

Immunohistochemistry is a powerful investigative tool that can provide additional information to the routine morphological assessment of tissues that are studied. Immunohistochemical techniques have adapted and been refined over time particular when used in fixed tissue. In this study standard IHC techniques were used to localise a number of cellular proteins within the endometrium of women undergoing UAE. The protocols were optimised for best immune-staining and least background staining.

2.3.1 **Antibodies**

Antibodies are host proteins found in plasma and extra cellular fluids that serve as the first response and comprise one of the principal effectors of the adaptive immune system. The body produces antibodies to annul foreign molecules & organisms. Since they are soluble, and secreted in large quantities, antibodies can be easily obtained & studied. The discovery of the ability of antibodies to bind to specific antigens like a lock and key mechanism led to their wide use in science disciplines and human healthcare has benefited from their use in developing diagnostic tests and therapeutic agents.
Antibodies are the secreted form of the B-cell receptor. They are also referred to as immunoglobulin (Ig) as they contain a common structural domain found in many proteins.
An antibody molecule consists of four polypeptides, two heavy chains and two light chains. These polypeptides join to form a Y-shaped molecule. This molecule consists of three equal-sized portions that are connected loosely by a tether.

There are five immunoglobulin classes of antibody molecules found in the serum: IgG, IgM, IgA, IgE and IgD. The Ig class determines both the type and the temporal nature of the immune response. All primary antibodies used in this research were of the IgG class. VEGF and COX-2 were polyclonal IgG while CD-34 was monoclonal.
Polyclonal antibodies are typically produced by inoculation of a suitable mammal with an antigen. The immune system instructs B-lymphocytes to produce IgG

immunoglobulins that are specific to that antigen. Larger mammals such as mice, rabbits and goats are used as the amount of serum that can be collected is greater. The polyclonal IgG is then purified and used. As polyclonal antibodies are produced by a variety of cells with each animal species, they can be immunochemically different and may react against various epitopes on the antigens they target. The epitope is a short amino acid sequence that the antibody is able to recognize as a part of the antigen.

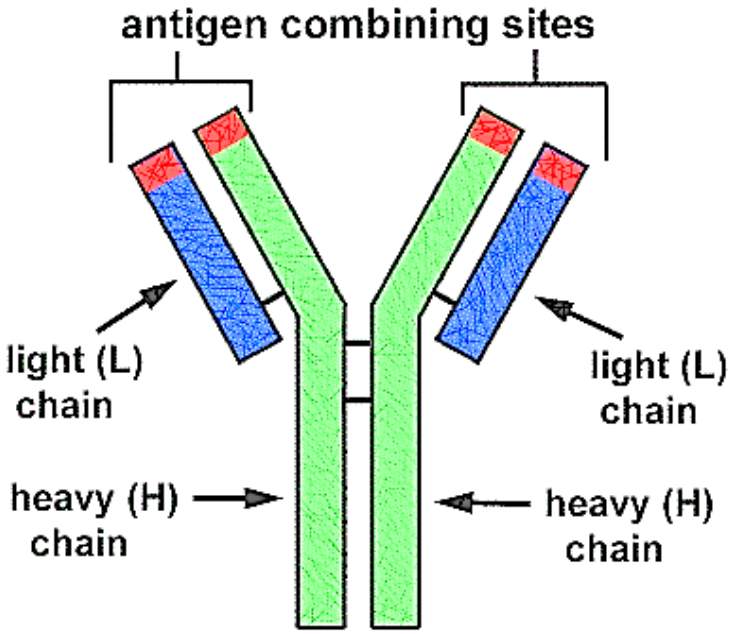


Figure 2-2: antibody structure

2.3.2 **Paraffin processing of tissue**

All endometrial tissue processing took place in the histopathology laboratory at Queen Elizabeth Hospital, Glasgow. This is a standard procedure that is performed by technicians in the laboratory.

The usual fixative for paraffin embedded tissues is 10% neutral buffered formalin (NBF). This is equivalent to 4% paraformaldehyde in a buffered solution plus a preservative (methanol) which prevents the conversion of formaldehyde to formic acid. Because of the preservative, NBF has a shelf life of months,

whereas 4% PF must be made fresh. Histological specimen pots are usually kept in all surgical departments & operating theatres in the NHS including the gynaecology department.

Optimal histology requires adequate fixation, about 48 hrs at room temperature for thinly sliced tissues. Inadequately fixed tissues will become dehydrated during tissue processing, resulting in hard and brittle specimens. Paraffin processing is then performed by taking the cassettes through a series of graded ethyl alcohol (ETOH) baths to dehydrate the tissues and then into xylene. Hot paraffin is then able to permeate the tissue. Processed tissues can be stored in paraffin blocks indefinitely at room temperature.

Immunostaining fixed specimens can be difficult as formaldehyde fixation may prevent recognition of epitopes by the primary antibody. Frozen sections are better. However, this would require repeating the immunostaining protocol after each specimen being taken.

2.3.3 **Tissue sectioning and mounting**

Paraffin embedded sample blocks of the study samples were collected from the pathology department archive at Glasgow Royal Infirmary and at the new pathology department archive at the Queen Elizabeth Hospital after obtaining the histopathology laboratory number. Sections were cut using a microtome (Leica RM 2135). Blocks to be sectioned were placed face down on an ice block for 10min. A fresh microtome blade was used and was replaced every 10 blocks. The microtome dial was set at 105μM until the cutting was smooth and then it was set at the desired 5μM. The slices were floated on a 37^{o}C water bath and then mounted on SuperFrost® glass slides (BDH, Merck House, and Poole). Slides were dried completely by vertically draining them at room temperature. The slides were then heated overnight in an oven at 45°C to achieve optimum section adherence to the glass.

2.3.4 Immunohistochemistry Avidin Biotin Complex (ABC) method

2.3.4.1 Deparaffinising and Rehydration

Slides must be deparaffinised and rehydrated prior to proceeding with any staining protocols. This process will allow antibodies to access the tissues. Poor staining of the section can be caused by incomplete removal of paraffin.
The slides were placed in a rack and were washed twice with Xylene for 3min. Then with Xylene1:1 with 100% ethanol for 3min. The slides were then put through ethanol baths (95%, 70% & 50%) for 3 minutes each. They were then rinsed with tap water and finally rinsed with 0.01 M phosphate buffered saline. From this point onwards the slides were kept wet. Drying out would cause non-specific antibody binding and therefore high background staining.

2.3.4.2 Antigen Retrieval

Most tissue that's fixed with formalin requires an antigen retrieval step prior to immunohistochemistry staining. The reason behind this is that methylene bridges form during fixation. These bridges cross-link proteins and can interfere with the recognition of epitopes on the antigen by the antibody.
The two methods of antigen retrieval are Heat-induced epitope retrieval (HIER) and proteolytic-induced epitope retrieval (PIER). We used either of these two methods to break the methylene bridges and expose the antigenic sites in order to allow the antibodies to bind. The temperature, the pH level and the time of incubation are all critical factors that must be controlled for optimal antigen unmasking without causing damage to the morphology of the section. Sodium citrate pH 6.0 or Tris/EDTA pH 8.0 buffers were used for microwaving sections in a pressure cooker.

2.3.4.3 Staining methods

An indirect staining method was used for all immunohistochemistry (IHC), were an enzyme labelled secondary antibody was directed against the primary antibody.
This allowed for amplification of the primary antibody-antigen complex thereby enhancing the target signal. The use of a biotinylated secondary antibody allows for subsequent incubation with pre-formed avidin-biotin complexes and can lead to further amplification of signal. Once these complexes attach to an enzyme-substance system, the amplified antibody-antigen complex can be visualised. This signal amplification due to the high affinity of avidin to biotin increases the sensitivity of staining techniques. This in turn helps to reduce unwanted background staining.

2.3.4.4 Controls

Validation of all IHC protocol was carried out by the inclusion of control tissue slides to ensure the primary antibodies worked as expected and specifically on their target. The inclusion of positive and negative controls was required. For negative controls, the primary antibodies were omitted. However, tissue samples which are known to express the protein of interest were used in the experiment for positive controls. (Table 2-3). This inclusion is required to ensure that the immunostaining achieved is within the expected cellular compartments.

Table 2-3: The used positive control tissues for different antigens in this study

Antigen	Positive control tissue
VEGF	Kidney
COX2	Colon
CD34	Colon
Ki67	Colon

2.3.4.5 Reducing background immunostaining

Before using antibodies to detect proteins by immunohistochemistry (IHC), all epitopes on the tissue sample should be blocked to prevent the nonspecific binding of the antibodies. Otherwise, the antibodies or other detection reagents may bind to any epitopes on the sample, independent of specificity. This step is essential prior to using antibodies to detect proteins by immunohistochemistry (IHC).

The blocking step for IHC is performed after sample preparation is completed and immediately prior to incubation with the primary antibody. The general protocol is to incubate the fixed, embedded, mounted, cleared and unmasked IHC sample with the appropriate blocking buffer for a time period from 30 minutes to overnight at either ambient temperature or 4ºC based on the optimized protocol specific to each antibody and target antigen. Adequate washing after the blocking step is critical to remove excess protein which may interfere with the detection of the target antigen.

Normal serum was the common blocking reagent as it carries antibodies that bind to reactive sites and thus prevents the nonspecific binding of the secondary antibodies used in the assay. It is vital to use serum from the species that the secondary antibody was generated in rather than that of the primary antibody. Serum from the primary antibody species would bind to reactive sites, but the serum from the secondary antibody would recognize those non-specifically-bound antibodies along with the antibodies bound to the target antigen. The prevention of peroxidase activity and reduction of background staining is achieved by submerging the section in hydrogen peroxide (H_2O_2).

Standard IHC techniques were performed to localise a number of cellular proteins within the human tissue. All protocols were optimised to determine the best conditions for maximal immunostaining with minimal background staining.

2.3.5 **General Immunohistochemistry Protocol**

Tissue section slides of 5µ thickness were placed in a rack and heated in an oven at 55°C for 35 minutes. Slides were then dewaxed by washing in xylene (2 x 10min) baths. They were then rehydrated by washing through graded alcohol concentration baths. This was by using 100% ethanol (2 x 5 min), 95% ethanol (2 x 5 min), 90% ethanol (2 x 5 min) and 70% ethanol (2 x 5 min). They were then washed in 0.01M phosphate buffered saline (PBS) pH 7.6 (5min x 1). Endogenous peroxidase activity was blocked by exposing the slides to 1.7% H_2O_2 in Methanol (300 ml Methanol + 5 ml H_2O_2) for 30 minute. The sections were then washed with PBS (2×10min).

Antigen retrieval was then performed by placing the buffer solutions in a pressure cooker (Lakeland Plastics Ltd.Cumbria, UK) for 15 min at full power to come to the boil. The slides were immersed in the buffer solutions and microwaved for 8min on full power. This takes into account that after 3 min the cooker should be at the required pressure and the slides are then under pressure for 5min. The sections were left to cool in the buffer solution for 20 minute. Sections were then washed in distilled water (1 x 5min) then again in PBS (1 x 5min).

The slides were then loaded into racks and placed in the auto-stainer (Leica XL) and covered with TBStw (Tris buffered saline/tween 20) buffer pH 7.6. A specific programme was setup on the machine for each antibody and was then chosen (section 3.5.4). Positive controls were used in every protocol to determine the best condition for maximal immunostaining with minimal background staining.

A counter staining protocol was then followed once the staining cycle was over. The slides were removed from the racks and counterstaining in Haematoxylin for 3 minutes and washed under running tap water for 1 minute. Acid alcohol was added for 5 seconds and then washed in tap water. This was followed by washing in Scott's tap water for 1 minute followed by a further wash in tap water. The slides were dehydrated for 1 minute in 95% Ethanol and then 1 minute in 100% ethanol then Xylene (3 x 5 min). Then mounted in DPX.

Sections were scanned and uploaded to Digital Slidebox by Roderick Ferrier, Laboratory manager & Clare Orange, TMA and Image Analysis Unit Manager at Glasgow University pathology department, Queen Elizabeth Hospital. Slide path software was used to access the slides and partly used for scoring.

Table 2-4 Summary of IHC protocols

Antigen	Primary antibody			Secondary antibody			Antigen retrieval	Negative control
	Species	Conc.	Incubation	Species	Conc.	Incubation		
VEGF-A	Goat	1:200	30min at 4°C	Horse-biotinylated	1:200	30min RT	0.01M Sodium Citrate	Omit Primary antibody
COX-2	Goat	1:500	30min at 4°C	Rabbit-biotinylated	1:200	30min RT	0.01M Sodium Citrate	Omit Primary antibody
CD34	Mouse	1:150	60 min at 25°C	Rabbit-biotinylated	1:200	30min RT	0.01M Sodium Citrate	Omit Primary antibody
Ki67	Rabbit	1:200	60 min at 25°C	Goat-biotinylated	1:200	30min RT	0.01M Sodium Citrate	Omit Primary antibody

2.3.6 **Scoring and analysis of immune-reactivity**

Semi-quantitative scoring systems are used to convert subjective perception of IHC-marker expression by the observer into quantitative data, which is then used for statistical analysis and drawing up conclusions. Without such a method, data would be provided with subjective perception and expressed as strong, weak, absent [172]. To reduce subjectivity it is recommended to have at least more than one observer when interpreting a clinical study [173].
The endometrial stromal tissue was scored. The immunostaining intensity of epitopes in VEGF and COX-2 antibodies stained sections was assessed in the semi-quantitative manner on the 4-point scale: 0= no immunostaining, 1= mild immunostaining, 2= moderate immunostaining, 3= intense immunostaining. The H-Score was derived by using the following equation:
% of mild staining x 1 + % of moderate stating x 2 + % of intense staining x 3

All tissue sections were scored by myself and one independent observer who was blinded to the patients' data. Coefficient correlation was accepted at ≥ 0.7.
The computerised image analysis system (Version 4.0, Digital Image Hub, Leica biosystems) was used to assess Ki67 expression & calculate microvascular density (MVD) when CD34 antibodies were used. The stained cells measuring algorithm was optimised and analysis was performed at 20X. This will be discussed in detail in section 3.5.6.

2.4 RNA Extraction

Due to time constraints, RNA extraction was performed by fellow researcher Dr Salha Abukhnjr . The extraction method was only observed, however, the analysis was performed by the author.
RNA isolation process was performed in a biosafety cold hood. All work surfaces and equipment needed such as pipettes, forceps and homogenizer were cleaned using either 75% Ethanol or Zap RNase

2.4.1.1 Isolation of RNA from frozen samples

Tissue samples were kept frozen on dry ice while weight measurement was undertaken. The cold cryo- pot was placed onto the balance and the balance was zeroed. The tissue sample was quickly added to the pot and weighed. Weight of samples taken was between 50 and 100mg. Large sample pieces were ground using a cold mortar, spatula and scrape with occasional addition of liquid nitrogen to keep cold. Samples were transferred to a glass tube including Trizol solution (1ml of Trizol was added to 50-100mg of tissue sample). Each sample was then homogenized using a hand holding homogenizer and separated into 1ml aliquots in autoclaved 1.5 Eppendorf tubes.
The tubes were then incubated in room temperature for 5 minutes to permit complete dissociation of nucleoprotein complexes. In order to keep a high RNA yield, 0.2ml of chloroform was then added to each 1ml Trizol and the samples were shaken vigorously. Tubes were incubated for 3 minutes at room temperature before they were centrifuged at 13,000 rpm for 15 minutes at 4 C°. The upper aqueous phase transferred to fresh autoclaved tubes and 0.5ml of Isopropyl alcohol was added to each 1ml Trizol used in the initial homogenization to precipitate RNA from the aqueous phase. A second centrifugation was performed at 13,000 rpm for 10 minutes at 4 C°. The tubes were then incubated for 10 minutes at room temperature. The isopropyl alcohol was decanted and a small white pellet was left in the bottom of the tube. 1ml of 75% ethanol was added to each tube then vortexed. At this stage some of sample was stored in - 80C°and the rest of the protocol continued later. The third centrifugation was done at 10,000 for 5 minutes at 4C°. Ethanol was poured off and tubes tapped carefully in order not to dislodge the pellet and left to dry for

10 minutes at room temperature. Appropriate amounts of DEPC water were added to each tube according to the pellet size (The range was between 40µl for a large pellet and 15 µl for a small one). Tubes were then vortexed, centrifuged quickly and incubated twice for 5 minutes at 65C°on a heating block. The tubes were kept on ice at all time during nanodrop measurement and then stored at -80C°.

2.4.1.1 Isolation of RNA from formalin –fixed paraffin- embedded samples

Extraction of RNA from formalin-fixed paraffin-embedded tissue (FFPE) was required for some samples where there was no fresh stored tissue. Many protocols have been described to extract RNA from FFPE samples. The first successful RNA extraction from FFPE samples was in 1988 [174]. In most of these protocols the RNA is extracted by spin column purification according to similar basic principles: deparaffinization, followed by cell disruption with heated proteinase K. After proteinase K incubation, RNA is isolated by alcohol precipitation [175].

The Ambien kit 1975 was used (recover all total nucleic acid isolation kit, Life technologies, Paisley, UK) and the accompanied protocol was followed. A histological section of 10-µm thickness was cut from each block using a conventional microtome. A separate blade was used for each block to prevent contamination. Each section was picked up by a cleaned forceps and placed into an Eppendorf tube. The section was then deparaffinazed by adding 1 ml of xylene to the tube, mixed then centrifuged at 10,000 rpm for 5 minutes at 4C. This was then followed by repeated addition of 1 ml absolute alcohol to the pellet, vortexed then centrifuged at full speed for 5 minutes. Alcohol was aspirated and discarded without disturbing the pellet. The pellet was allowed to air dry at room temperature for 10 min before the addition of lysis buffer. 400µl of digestion buffer and 4µl of protease were added to the samples (from the kit).

The tubes were mixed gently, then placed into the rotatory oven at 50C°and incubated with rotation for 3 hours. Proteinase K incubation at high temperature is capable of efficiently degrading proteins that were covalently cross-linked with each other and RNA, thereby allowing more efficient RNA extraction [176].

After proteinase K incubation, RNA was isolated by quantification and qualification of RNA by adding 480µl additive solution from the Ambion kit. At this stage, a filter cartridge was placed into one of the collection tubes supplied in the kit.

The mixed solution was pipetted into the filter cartridge and centrifuged at 10,000 rpm for 60 seconds to pass the mixture through the filter and the flow through solution discarded. The filter cartridge was then washed by 700µl and 500µl of wash 1 and wash 2/3 solution respectively. DNase mix was added to the centre of the filter, where the mix including DNase buffer 6µl, DNase 4µl and nuclease free water 50µl. A master mix was made when more than one sample was being processed.

The washing process was repeated after 30 minutes incubation at room temperature once with 500µl of wash 1 and washed twice with 500µl wash 2/3. The filter cartridge was transferred to a fresh collection tube and 15µl of nuclease free water, heated to 95C°, was added to the centre of the filter. The tube was then incubated for 60 seconds at room temperature then centrifuged for 1 minute at 13,000 to pass the mixture through. The collected sample volume was checked by the Nanodrop and stored at -80C°.

2.4.2 **RNA quantification and qualification**

The quantity of total RNA extracted was measured using an automated spectrophotometer (RNA 6000 Nanodrop). The concentration of total RNA in an aliquot of 1µl of sample was determined in µg/µl and the ratio of optical density at a wavelength of 260nm to a wavelength of 280nm (260:280) was additionally calculated. The sample was considered not to have sufficient purity for use in further work when it had a 260:280 ratio of less than 1.6. Quality and integrity of the extracted RNA was examined by Agilent 2100 Bioanalyser system. This was as a paid service performed by the Biochemistry Department, University of Glasgow (Glasgow Biomedical Research Building). Only RNA that displayed intact 18S and 28S peaks was reverse transcribed (RT) to cDNA for real-time PCR analysis. RNA integrity number (RIN) for the tested samples with clear 18S, 28S peaks was between 3 to 9.

2.4.3 **Selection of endogenous control gene**

This was based on previous work by Dr Salha Abukhnjr were B-actin was found to have the narrowest standard deviation compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18S.

The primer probes used for gene expression and quantification of human ACTB (B actin) and vascular endothelial growth factor-A (VEGF-A) were purchased as predesigned, inventoried Spans exons Taqman Gene Expression Assays from Life Technologies (table2-5). Quantification was implemeneted on an Applied Biosystems 7900 using a 96 well plate. The thermal cycler conditions were 50^{o}C for 2 minutes, 95^{o}C for 10 minutes followed by 40 cycles of 95^{o}C for 15 seconds and 60^{o}C for 1 minute.

Table 2-5: Target assay mixed and endogenous control probe in QPCR

Gene Symbol	Gene Name	Assay ID	Supplier
B actin	Human ACTB (B actin) endogenous control	4310881E	Life technologies
VEGF-A	Vascular endothelial growth factor A (VEGF-A)	Hs00900055>m 1	Life technologies

2.5 Magnetic Resonance Imaging (MRI) study

2.5.1 **Measuring the dominant fibroid:**

Sagittal T2 4MM and Coronal T2 4MM were used to measure the dominant fibroid. The anterior-posterior (A-P) diameter and the longitudinal diameter of the fibroid were measured in centimetres on the sagittal T2 4MM images. The transverse diameter of the fibroid was measured on the coronal T2 4MM images. The MRI slice where the fibroid was of maximum size was used. Careful attention was taken to the placement of the measurement callipers when measuring the fibroid and inclusion of non-fibroid uterine tissue was avoided (Fig 2-3). In certain cases where the margin/plain between the fibroid tissue and the uterine tissue was not easily identified, contrast enhanced images (sagittal & coronal LAVA C+) was used to accurately assess the margin as these MRI sequences produce a marked contrast difference between fibroid and uterine tissue (fig 2-4).

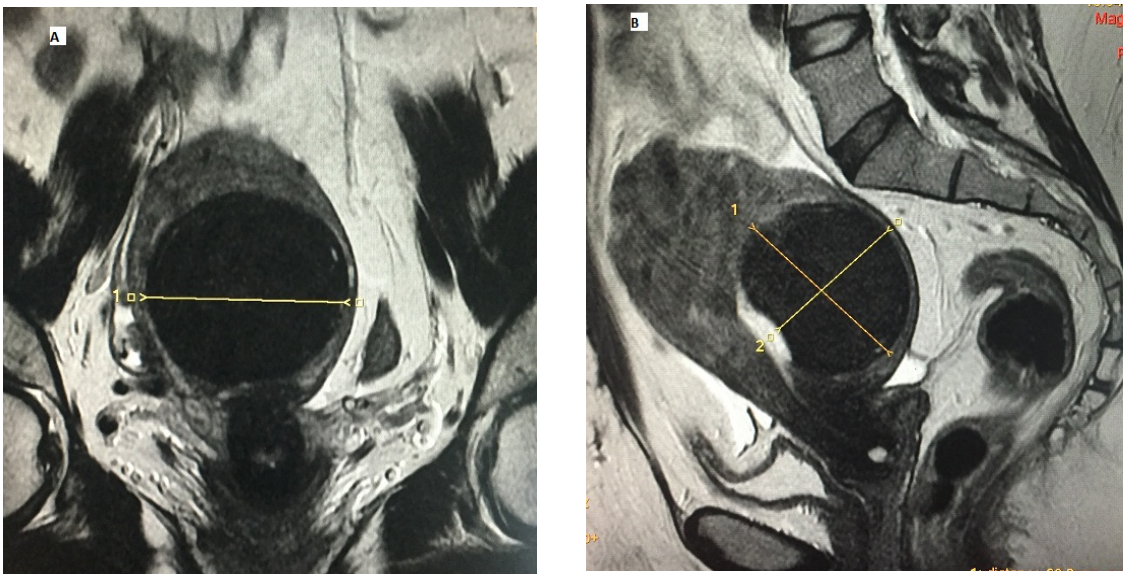


Figure 2-3: T2 weighted MRI pelvis. Image A (left) shows a transverse diameter of a fibroid measured on a coronal section MRI slice. Image B Right) shows A-P & longitudinal diameters measures on a transverse section MRI slice.

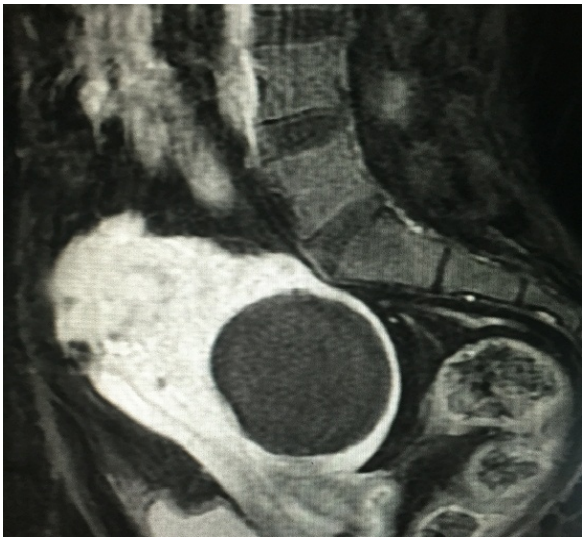


Figure 2-4 Contrast enhanced MRI pelvis showing marked plane between fibroid tissue and the myometrium post-UAE

2.5.2 **Measuring the fibroid uterus:**

A similar approach using sagittal T2 4MM and coronal T2 4MM was used to measure the fibroid uterus in centimetres. The A-P diameter and the longitudinal diameter of the uterus was measured on the sagittal T2 4MM images. The transverse diameter of the uterus was measured on the coronal T2 4MM images. The slice where the uterus was of maximum size was used and this measurement incorporated the fibroids. In cases of a pedunculated fibroid that had a stalk then the uterus was measured without incorporating the fibroid measurements.

2.5.3 **Calculating the volume of the fibroid and uterus**

The volume of each fibroid was calculated in cm^3 using the standard prolate ellipse formula AP x CC x TR x 0.523 [177]. A similar method was used to measure the uterine volume. We also sub grouped the above according to the volume and looked at the response following UAE. (section 4.10.2)

2.5.4 **Assessing the endometrium:**

In some analysis in chapter 5 the endometrial thickness was measured in millimetres in a mid-sagittal slice using the sagittal T2 4MM sequence. The normal endometrium is homogeneously hyper-intense on T2 weighted imaging, regardless of the phase of the menstrual cycle and well outlined by the low signal myometrial junctional zone.
The measurement was performed by placing the callipers from the basal endometrial surface across the endometrial canal (cavity) to the opposite basal surface. Care was taken not to include the myometrium in the measurement by zooming the relevant section of the MRI image up to x3 zoom. Although the timing of the period was not recorded at the time of MRI, it was thought that significant changes to the endometrial thickness would still be recognizable.

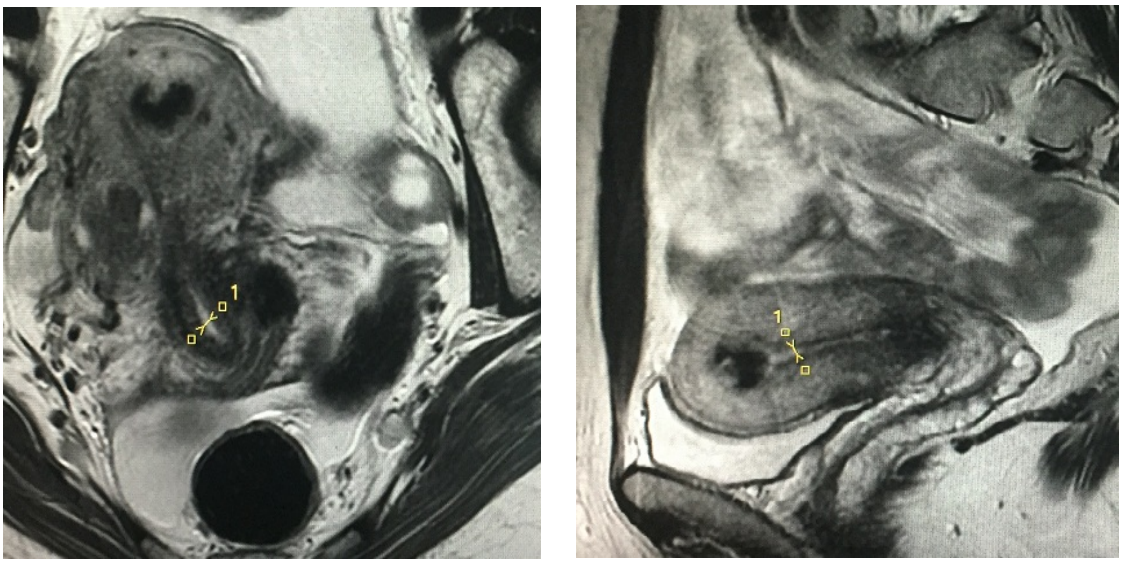


Figure 2-5 T2 weighted MRI of the uterus with callipers used to measure the endometrial thickness. Coronal section (left) and sagittal section (right).

2.5.5 **Diffusion weighted MRI**

We will discuss in detail later in chapter 5 the concept of diffusion weighed MRI and how to assess damage to cell membrane through measuring the ADC values (Apparent Diffusion Coefficient). Initially, our aim was to measure this for all the patients in the study. However, we soon realised that there is a protocol variation between the different hospital sites that provide MRI facilities within

NHS Greater Glasgow and Clyde. The make of the MRI machines were also different. Therefore we only measured the ADC value of 20 patients in a retrospective manner. These patients were involved in a study that compared different embolic agents. The advantage of this is that all patients received DWMRI pre-UAE; 24hour post UAE & 6 months post UAE. They were all performed in the same hospital, the same MRI machine and a standardised MRI protocol. Only under these circumstances would the results of ADC calculations and analysis be valid. (section 5.7)

2.5.6 **Pictorial blood assessment chart (PBAC)**

The participants in the histological studies were given a PBAC diary to fill in prospectively for one period prior to the UAE procedure (fig 2-6). The scoring method used to evaluate the PBAC score of the diary was not revealed to the patients. A further PBAC diary was sent to the patients to fill in by post 6 months from the day of UAE fig (2-7).
The PBAC diary consists of a series of diagrams representing lightly, moderately and heavily soiled tampons or towels. The participant would mark the appropriate box at the time that each sanitary product was discarded. In addition, passage of clots (size equated to that of 1p and 5p coinage) and episodes of flooding were recorded. The diaries where collected and scored as follows:

Towels: A lightly stained towel will score 1 point, a moderately stained towel will score 5 points and a towel which is fully saturated with blood will score 20 points.

Tampons: A lightly stained tampon will score 1 point, a moderately stained tampon will score 5 points and a tampon which is fully saturated with blood will score 10 points.

Clots and Flooding: Clots were divided into 1p size which score 1 point and 50p size which would score 5 points. Flooding was scored as 5 points.

Each individual PBAC chart was scored and a score of 100 or greater indicated HMB. The capacity to absorb blood varies widely between mini and a super plus tampons and the same goes for different towels (with and without wings). Therefore the accuracy of this method may be limited due to the wide variety of sanitary products in the retail market. Despite our attempt to address this issue by including a space on the PBAC diary for the brand and variant of the sanitary products used, not all the women in this study filled this section.

TOWEL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Clots								
Flooding								
TAMPON	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Clots								
Flooding								

Figure 2-6: PBAC Chart

TOWEL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	12	6						
			4					
				2-3	2-3	1		
Clots								
Flooding	✓	✓						
TAMPON	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Clots								
Flooding								

360
20
397

Figure 2-7: Example of a completed PBAC Chart

2.6 Statistics

Descriptive statistics were used when it was appropriate. TaquMan data were collected on Microsoft excel sheet for RNA analysis. Mann-Whitney test was used to compare between groups for non-parametric data and results expressed in median values with a 95% confidence interval. Paired t-testing was used to compare parametric data and the results expressed in mean +/- standard deviation. One way ANOVA test used when appropriate and Pearson's & Spearman Rho correlation coefficients were used for association between variables after testing for normality.

Results were statistically significant with p value ≤ 0.05. Details of when each test was used are addressed in each relevant chapter under the statistics/results section. Minitab 17 statistical software was used for data analysis and creating graphs that were then edited using Microsoft word 2010 software. Microsoft excel 2010 was used to draw graphs.

3. Chapter 3: Laboratory

The expression of VEGF-A, COX 2 & Ki67 and the microvascular density in the endometrium of women with fibroids undergoing uterine artery embolisation

3.1 Vascular Endothelial Growth Factor

3.1.1 Introduction:

Throughout each menstrual cycle, the endometrium undergoes remarkable changes from regeneration and growth to apoptosis and shedding. These cyclic endometrial modifications are controlled by the variations of sex steroids concentrations and the discontinuation of ovarian steroid support will trigger a dynamic array of events characteristic of menstruation. New vascular support develops via angiogenesis, vascular remodelling in the endometrium during each menstrual cycle. This in turn supports the regeneration and growth of endometrial cells. [34]

This vascular remodelling is regulated by several angiogenic factors in the human endometrium [178]. One of these factors is vascular endothelial growth factor (VEGF). It stimulates endothelial cell proliferation, permeability, migration and assembly into capillary tissue. The role of VEGF in early developmental angiogenesis is emphasized by the finding that the loss of a single VEGF allele will result in defective vascularization and early embryonic demise. [179]

The proliferation of the endometrium is a result of neovascularisation and this has been shown to be directly related to increases in VEGF [180]. Blockade of VEGF using a potent VEGF blocker completely inhibits neovascularisation during endometrial regeneration and has no marked effect on pre-existing or newly formed vessels. This suggests that VEGF is essential for neoangiogenesis but not the survival of mature vessels in the endometrial vascular bed [181].

3.1.2 VEGF isoforms and receptors

VEGF is a secreted heparin-binding polypeptide mitogen with a target cell specificity restricted to vascular endothelial cells. In vivo it induces microvascular permeability and plays a central role in angiogenesis and vasculogenesis. Through alternative mRNA splicing, a single gene gives rise to five isoforms of VEGF. These are identified according to their amino acid residue length (121/120, 145, 165/164, 189/188, 206) Fig 3-1. The two shorter ones are efficiently secreted while the longer ones are mostly cell-associated which suggests multiple physiological roles for this family of polypeptides. [182] [183]

VEGF 165 is the predominant isoform while VEGF145 and VEGF189 are the less frequently reported splice variants [16, 179]. Two VEGF receptor tyrosine kinases (VEGFRs) have been identified, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 seems to mediate almost all observed endothelial cell responses to VEGF, whereas roles for VEGFR-1 are more elusive. VEGFR-1 might act predominantly as a ligand-binding molecule, sequestering VEGF from VEGFR-2 signalling. Several isoform-specific VEGF receptors exist that modulate VEGF activity. Neuropilin-1 acts as a co-receptor for VEGF165, enhancing its binding to VEGFR-2 and its bioactivity. Heparan sulphate proteoglycans (HSPGs), as well as binding certain VEGF isoforms, interact with both VEGFR-1 and VEGFR-2. HSPGs have a wide variety of functions, such as the ability to partially restore lost function to damaged VEGF165 and thereby prolonging its biological activity. [182]

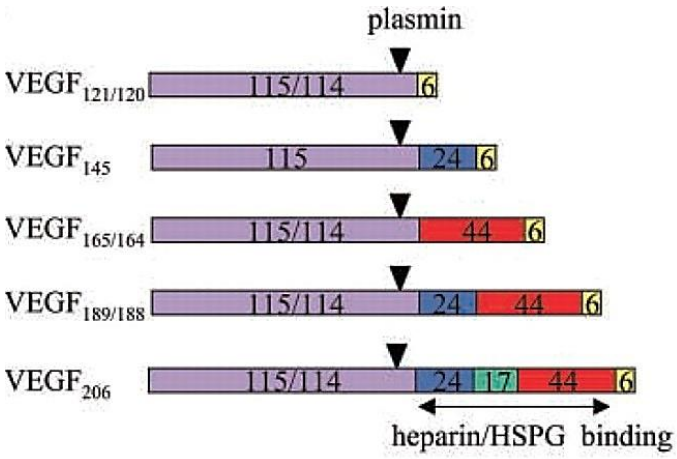


Figure 3-1: Splice variants of human VEGF.

3.1.3 **VEGF in the endometrium**

Following shedding at menstruation, the human endometrial functional layer regrows from the basal layer during the proliferative phase of the menstrual cycle, increasing several-fold in thickness over the space of a few days. Such remarkable growth is accompanied by expansion of the vascular tree involving angiogenesis and arteriogenesis. Significant arteriogenesis continues during the secretary stage of the cycle, with growth and coiling of the endometrial spiral arterioles [184]. Hypoxia, after menstruation enhances angiogenesis by inducing human stromal cells to express VEGF [185]. VEGF regulates the growth and

differentiation of the endometrium during the menstrual cycle. This is by regulating the epithelial and stromal development in the endometrium under the influence of oestrogen and progesterone [181]. However one study has shown that hypoxia is not required for human endometrial breakdown or repair in a xenograft model of menstruation [186].
Abnormal bleeding is a result of unrestrained angiogenesis leading to large fragile endometrial vessels. Aberrant angiogenesis therefore may underlie abnormal menstrual bleeding associated with uterine fibroids. The normal menstrual cycle haemostasis and menstruation is a well-ordered and highly regulated process. In contrast to this, heavy menstrual bleeding reflects a derangement in these physiological processes and the levels of VEGF [187] .

Oestrogen has been found to stimulate VEGF production in the endometrium and may have a role in promoting fibroid growth by stimulating angiogenesis [188]. Ovarian sex steroids are known to play a role in the proliferation and maintenance of uterine leiomyomas. Therefore, a state of low oestrogen such as that in menopause is associated with the shrinkage of uterine fibroids.

A similar state can be obtained with the use of Gonadotrophin releasing hormone agonist (GnRH-agonist). This reversibly suppresses the ovarian function, lowering the oestradiol level to a menopausal level and therefore reduces fibroid size [189]. GnRH-agonist therapy is used preoperatively in premenopausal women with fibroids awaiting surgery to reduce the size of the fibroid uterus. It has been shown that once GnRH-agonist treatment is stopped, uterine fibroids return to their original size [190].

Many studies, whether histochemical or PCR studies, have focused on the patterns of VEGF-A gene expression in human endometrium in normal and heavy menstrual bleeding. Therefore treatment of fibroids should theoretically alter the level of VEGF. This study focuses on the patterns of VEGF-A and its main receptors gene expression in the endometrium of women with heavy menstrual bleeding secondary to uterine fibroids and how this changes post UAE.

3.1.3.1 Unpublished VEGF work

In this section we will look at unpublished research that was undertaken by Dr Salha Abukhnjr at the University of Glasgow looking at the patterns of expression of VEGF in the endometrium of women suffering from HMB with and without uterine fibroids. With her permission we will summarise the results.

In women with HMB and normal uteri, immunohistochemistry showed that VGEF expression in the endometrium was highest in the menstrual phase and lowest during the early secretory phase. There was significant differences between the two levels (95% Cl (129.99, 30.00) P= 0.019). The expression of VEFG-A was higher in the proliferative phase than the early secretory phase (95% Cl (99.97, 0.02) p=0.04). When using RT-PCR, the VEGF-A mRNA expression in the endometrium varied significantly across the menstrual cycle (p=0.006). No difference in the expression of VEGFA was found between the different menstrual phases.

In women with uterine fibroids, the pattern of VEGF-A expression in the endometrium increased across the menstrual cycle with no significant difference between the different phases. The RT-PCR showed that the relative VEGF-A mRNA expression was significantly higher in the proliferative phase and mid-late secretory phase than the early secretory phase (P= 0.008, p=0.009, respectively, Mann-Whitney test). There was no difference between the proliferative and mid-late secretory phase.

However, there was no difference in VEGF-A expression in the endometrium throughout the menstrual cycle between women with HMB and normal uteri and women with HMB and uterine fibroids except in the early secretory phase (97% Cl (30.03, 0.00), $p<0.05$). This was higher in endometrial samples for women with uterine fibroids. RT-PCR data found no difference in the expression of VEGF-A in endometrium of women in these two groups.

3.2 Cyclooxygenase-2

In women suffering from HMB significant expression of COX-2 in the endometrium has been demonstrated when compared to woman with normal menstrual blood loss [25]. A positive relationship between the volume of blood loss and COX-2 in the endometrium of women with HMB has been previously demonstrated [191].

COX-1 is an enzyme expressed in nearly all body tissues and generates PGs for normal physiological function and inflammatory response. However, there have been studies that link the development of endometrial carcinoma to upregulation in the expression of COX1 [192]. COX2 has also been identified as having a role in different pathological conditions such as rheumatic diseases, inflammation and tumorigenesis [193].

Although PGs play a primary role as mediators in diseases such as inflammation and cancer, they also play a crucial role in normal physiological functions such as that of the female reproductive tract [194]. Reproduction in mice deficient in COX1 and COX2 were studied. The results showed that COX1 plays a role in determining the normal gestational period and parturition while COX2 plays a role in ovulation, fertilization and implantation. Any reduction in COX2 was seen to be detrimental to these processes [194-197]. In the human female, dysregulation in endometrial prostanoids can cause menstrual disorders such as HMB [31, 198].

The process of normal menstruation is under the influence of the sex steroid hormones by which cyclical breakdown and remodelling of the endometrium occurs. Towards the end of the menstrual cycle and at the time of progesterone withdrawal, COX2 expression is upregulated in the functional layer of the endometrium [23, 24, 199] with subsequent synthesis of PGE2 and PGF2α (a vasoconstrictor) and an increase in the expression of prostaglandin E2 receptors [200, 201].

The expression of COX-2 in the endometrium of women with uterine fibroids before and after uterine artery embolisation will be studied in this thesis.

3.2.1.1 Unpublished COX2 work

In this section we will look at unpublished research that was undertaken by Dr Salha Abukhnjr at the University of Glasgow looking at the patterns of expression of COX-2 in the endometrium of women suffering from HMB with and without uterine fibroids. With her permission we will summarise the results.

In women with HMB and normal uteri (without uterine fibroids), immunohistochemistry showed that COX2 expression in the endometrium was higher during the mid-secretory than the menstrual phase (2.3±0.3, 1.2±0.2 respectively, $p<0.05$). However, in women with HMB who had uterine fibroids, there were no significant differences in the expression of COX2 across the menstrual cycle but wide infiltration of COX2 protein was found in the fibroid tissue.

3.3 Microvessel Density (MVD)

Microvessel density is a measure of the number of vessels per high power (microscope) field and therefore reflects inter-capillary distance. Oxygen and nutrient consumption limit how far away from the vasculature tumour cells can remain viable.

Quantification of tumour vasculature may provide an indication of tumour angiogenic activity. One often-quantified aspect of tumour vasculature is micro-vessel density. It has been used in a wide variety of cancers as a prognostic indicator but has not been much used in benign tumours such as fibroids [202].

There have been studies that have looked at the relationship between MVD and the levels of angiogenic factors such as VEGF [203]. *Goteri et al* found no correlation between VEGF expression and an increased MVD in the endometrium. However, the study was looking at women suffering with adenomyosis and not uterine fibroids [204].

Contrary to the normal belief MVD does not reflect angiogenic activity or angiogenic dependence of a tumour [205]. MVD may serve as a surrogate marker for the efficacy of antiangiogenic therapy and success of treatment. UAE is considered a global antiangiogenic therapy as it blocks the main blood supply to the uterus by interrupting the blood flow through the uterine arteries. Although most research in this field is directed towards malignant tumours, a great deal can be learnt from studying non-malignant tumours such as fibroids.

MVD has not been widely utilized in the field of gynaecology oncology nor benign gynaecology. Measurement of MVD has been used as a prognostic indicator for human breast and prostate carcinomas. This helps in assessing the stage of disease, survival rate, recurrence rate and planning the treatment course [205].

Changes in MVD of the endometrium can appear very early once exposed to progesterone. This can be as early as 3 weeks after exposure [206]. Rogers et al found that MVD in the endometrium is higher in women with levonorgestrel implants than controls and that bleeding occurred despite having thin endometrium on ultrasound scan [207]. *Czekierdowski et al* found a significant difference in MVD between normal endometrium and that showing cancerous changes [208].

To date, there have been no human studies that looked at MVD before and after UAE. *Tan et al* described a study on female guinea pigs undergoing UAE and found that UAE causes a temporary decrease in MVD which reverses over time and none of the guinea pigs had a fibroid uterus [209].

We measured the change in MVD in the endometrium before & after UAE to assess if there is any significant change after exposing the uterus to an anti-angiogenic treatment such as UAE.

3.4 Ki67

Ki67 is a monoclonal antibody and a cellular marker for proliferation and its expression is strictly associated with cell proliferation. It is unique in that its antigen is absent from resting cells (G0) and is present in all active phases of the cell cycle (G1 & G2) [210] (fig 3-2). *Gerdes et al* first used Ki67 to determine the proportion of proliferating cells in malignant non-Hodgkin's lymphoma in 1984 [211]. The name was derived from the city of origin (Kiel) and the number of original clone in the 96-well plate. It was first thought to be an antigen but later found to be a protein[210]. Further research made it evident that Ki67 antibody could be used to estimate the growth fraction in any human cell population.

It has been shown that secretory endometrium that showed increased Ki67 expression can be used to aid the diagnosis of endometrial hyperplasia [212]. There have been studies looking at Ki67 in endometrial carcinoma and comparing it to normal endometrium. They concluded that high Ki67 expression correlates to morphologic features of aggressiveness of cancer. On the other hand, in normal endometrium, its expression pattern corresponded to the time of the period the endometrium was collected at [213].

There may also be a role for Ki67 expression in assessing endometrial proliferation in women with fertility and conception disorders. Insufficient cell proliferation of the endometrium has been linked to recurrent miscarriage and implantation failures [214]. Such dysregulation in endometrial proliferation may explain subfertility in women with uterine fibroids.

As the human endometrium undergoes a proliferative phase throughout the menstrual cycle it was felt appropriate to measure the expression of Ki67 in the endometrium before and after UAE. A treatment modality that reduces menstrual blood loss may lead to a detectable change in the expression of Ki67 that could partly explain the mechanism of how it works.

Although the levels of expression of Ki67 have not been studied in the various stages of the human endometrium, they have been studied in rodents. The expression was found to change according to the stage of the rodent estrous

cycle [215]. To date there has been no study looking at the change in Ki67 expression in the endometrium before and after UAE and this may serve as a pilot study paving the way for more research.

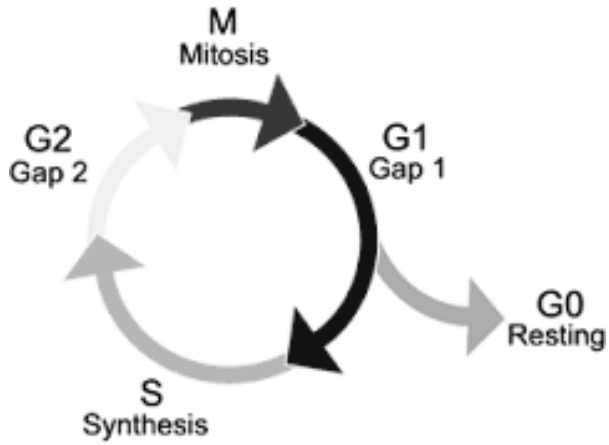


Figure 3-2: The cell cycle

3.5 The method

3.5.1 Subjects

The subjects studied were women aged between 42 – 52 years who presented to the tertiary interventional radiology outpatient clinic in Gartnavel general hospital and the Gynaecological Outpatients service, Glasgow Royal Infirmary. These women complained of heavy menstrual bleeding with known uterine fibroids diagnosed by ultrasound or MRI. Initially recruitment was for subjects that had not received any hormonal preparation in the last three months preceding biopsy collection. However, recruitment was poor therefore patients who were on hormonal treatment were also recruited into the study. It was felt to be unethical to ask a patient to stop hormonal preparations for the study.Ethical approval had previously been obtained from the West of Scotland REC 5 research ethics committee and a major amendment was submitted and approval was granted (appendix 1). A patient information leaflet was given to each patient and allowed a minimum of one week prior to deciding to join the study. Written informed consent was obtained from each patient before tissue collection and menstrual blood loss was assessed using the Pictorial Blood Loss Assessment Chart (PBAC) as in chapter 6.

3.5.2 Power calculation:

We aimed for a power of 90% or equivalent with a type II error probability of 2.0. UAE in women with HMB secondary to uterine fibroids leads to a reduction in menstrual blood loss of about 125ml after 6-9 months following treatment [216]. We sought help from Dr David Young, statistician, NHS advisor.

Table 3-1: Power calculation

Parameter	Power	Pre-UAE & post-UAE difference	SD	Sample size	Reference
Effect of UAE	90%	60% difference	1	22	*Khaund et al* [216]

3.5.3 **Tissue collection**

Endometrial samples were obtained by means of endometrial sampling using a Pipelle® sampler. The first sample was taken pre-UAE and the second was taken 6 months post-UAE. See section 2.2.1-2.2.2 for biopsies handling and storage. Endometrial biopsies were dated according to Noyes criteria (Noyes et al., 1975) by a single pathologist (Prof. Alistair Williams, University of Edinburgh) and using the reported last menstrual periods (LMP) (table 3.1). Circulating oestradiol and progesterone concentrations were measured at the time of endometrial biopsies and were consistent with the histological assessment (Table 3-2).

Table 3-2: Summary of endometrial biopsies collected from women with uterine fibroids both pre and 6 months post UAE according to phase of cycle

Histological dating of the sample	No. of samples
Proliferative	10
Early secretory	3
Mid Secretory	6
Inactive endometrium	6
Inadequate for staging (insufficient sample or endometritis)	6
Chronic endometritis	2
Non endometrial samples (cervical)	2
Total =	36

Table 3-3: Summary of blood samples for hormonal profile

Stage of the endometrium	Blood samples from women with uterine fibroids and HMB			
	Mean oestradiol (range)(pmol/l)	Mean progesterone (range) (nmol/l)	Mean LH (range) (nmol/l)	Mean FSH (range) (nmol/l)
Proliferative	729	< 2.0	16.4	9.2
Early secretory	468	3.2	22.3	14.4
Mid secretary	167	5.1	3.3	3.8
inactive	126	<2.0	< 0.5	< 0.5

3.5.4 Immunohistochemistry

3.5.4.1 VEGF-A immunohistochemistry

To investigate the expression of the VEGFA in human endometrium tissue before and after uterine artery embolisation, the standard immunohistochemistry protocol was followed to rehydrate sections (section 2.3.5). Human kidney was used as a positive control (fig 3-3 & 3-4). The programme for the auto-stainer was as follows:

1-Place slides in a humid staining chamber and cover with TBS buffer pH7.6, to which Tween 20 has been added. Leave for 5 min.
2-Drain off TBS buffer; wipe excess from around section
3-Incubate with 2.5% normal horse serum (NHS) for 20 min provided in the ImPRESS kit (Vector) together with the ImPRESS link reagent.
4-Drain NHS from sections without washing and wipe excess from around sections.
5-Incubate in biotinylated anti goat VEGF-A at 1:200 for 30 mins, diluted in buffer (10% horse serum TBStw)
6-Rinse thoroughly with TBS buffer pH 7.6, Tween 20 for 3x 5min.
7-Incubate sections in biotinylated anti-horse 1:200 for 30 min and wash slides in diluted in 10% horse serum TBStw (buffer) for 5 min.
8-Incubate section with Streptavidin ABC reagent (Goat IgG) for 30min then wash with buffer
9- Wash with TBStw then with Dako DAB – made from Vector DAB kit (DAB, DAKO Corp, Ca, USA). One drop substrate added to 1ml diluent (1 x 10 min). Rinse in distilled water and place in running tap water for at least 5 min.
10-The counter staining protocol was then followed as (section 2.3.5).
Sections were scanned and uploaded to Digital Slidebox

3.5.4.2 COX-2 immunohistochemistry

To investigate the expression of the COX2 in the human endometrium before and after uterine artery embolisation, the standard immunohistochemistry protocol was followed (2.3.5). Human colon was used as positive controls for optimising the immunohistochemistry protocol (fig 3-6). The programme for the auto-stainer was as follows:

1-Place slides in a humid staining chamber and cover with TBS buffer pH7.6, to which Tween 20 has been added. Leave for 5 min.

2-Drain off TBS buffer; wipe excess from around section

3-Incubate with 2.5% normal horse serum (NHS) for 20 min provided in the ImPRESS kit (Vector) together with the ImPRESS link reagent .

4-Drain NHS from sections without washing and wipe excess from around sections.

5-Incubate in biotinylated anti goat COX-2 at 1:500 for 30 mins, diluted in buffer (10% horse serum TBStw)

6-Rinse thoroughly with TBS buffer pH 7.6, Tween 20 for 3x 5min.

7-Incubate sections in biotinylated anti-rabbit 1:200 for 30 min and wash slides in diluted in 10% horse serum TBStw (buffer) for 5 min.

8-Incubate section with Streptavidin ABC reagent (Goat IgG) for 30min then wash with buffer

9- Wash with TBStw then with Dako DAB – made from Vector DAB kit (DAB, DAKO Corp, Ca, USA). One drop substrate added to 1ml diluent (1 x 10 min). Rinse in distilled water and place in running tap water for at least 5 min.

10-The counter staining protocol was then followed as (section 2.3.5).

Sections were scanned and uploaded to Digital Slidebox

3.5.4.3 Ki67 immunohistochemistry

To investigate the expression of the Ki67 in the human endometrium before and after uterine artery embolisation, the standard immunohistochemistry protocol was followed (2.3.5). Human colon was used as positive controls for optimising the immunohistochemistry protocol (fig 3-8). The programme for the auto-stainer was as follows:

1-Antigen retrieval using Menarini antigen access unit, EDTA buffer pH8

2-Peroxidase block (ThermoFisher) for 10 min

3-Incubate with 2.5% normal horse serum (NHS) for 20 min provided in the ImPRESS kit (Vector) together with the ImPRESS link reagent .

4-Drain NHS from sections without washing and wipe excess from around sections.

5-Incubate with 1:200 anti-Rabbit Ki67 for 45min

6- Incubate sections in biotinylated anti-goat 1:200 for 30 min and wash slides in diluted in buffer for 5 min

7-Incubate in VECTASTAIN™ Elite ABC Peroxidase reagent (rabbit IgG) for 30min then wash with buffer

8- Wash with TBStw then with Dako DAB

9- The counter staining protocol was then followed as (section 2.3.5).

Sections were scanned and uploaded to Digital Slidebox

3.5.4.4 CD34 immunohistochemistry

To measure MVD in the human endometrium before and after uterine artery embolisation, the standard immunohistochemistry protocol was followed (2.3.5). Human colon was used as positive controls for optimising the immunohistochemistry protocol (fig 3-7). The programme for the auto-stainer was as follows:

1-Antigen retrieval using Menarini antigen access unit, EDTA buffer pH8

2-Peroxidase block (ThermoFisher) for 10 min

3-Incubate with 2.5% normal horse serum (NHS) for 20 min provided in the ImPRESS kit (Vector) together with the ImPRESS link reagent.

4-Drain NHS from sections without washing and wipe excess from around sections.

5-Incubate with 1:150 anti-mouse CD34 for 60min at 25^{o}C

6- Incubate sections in biotinylated anti-rabbit 1:200 for 30 min and wash slides in diluted in buffer for 5 min

7-Incubate in VECTASTAIN™ Elite ABC Peroxidase reagent (mouse IgG) for 30min then wash with buffer

8- Wash with TBStw then with Dako DAB

9- The counter staining protocol was then followed as (section 2.3.5).

Sections were scanned and uploaded to Digital Slidebox

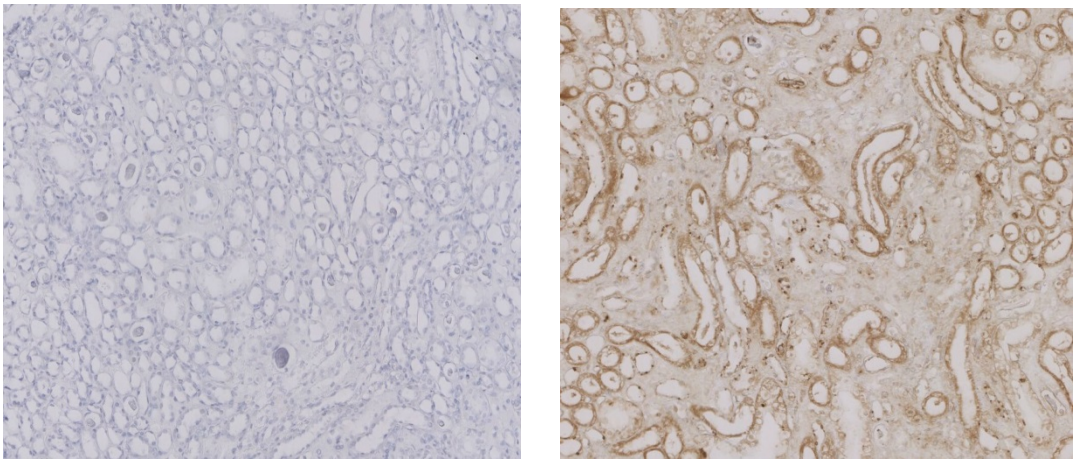


Figure 3-3: Human kidney tissue was used as a positive control for VEGF-A IHC. Pre-staining (left) & post staining (right)

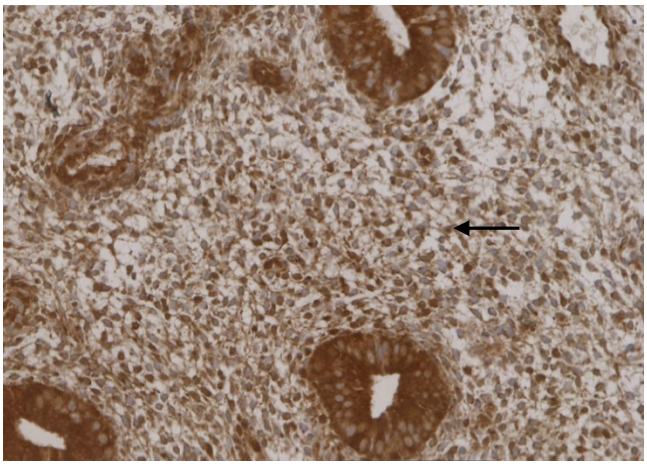


Figure 3-4: Strong expression of VEGF-A protein in the endometrial stroma (arrow)

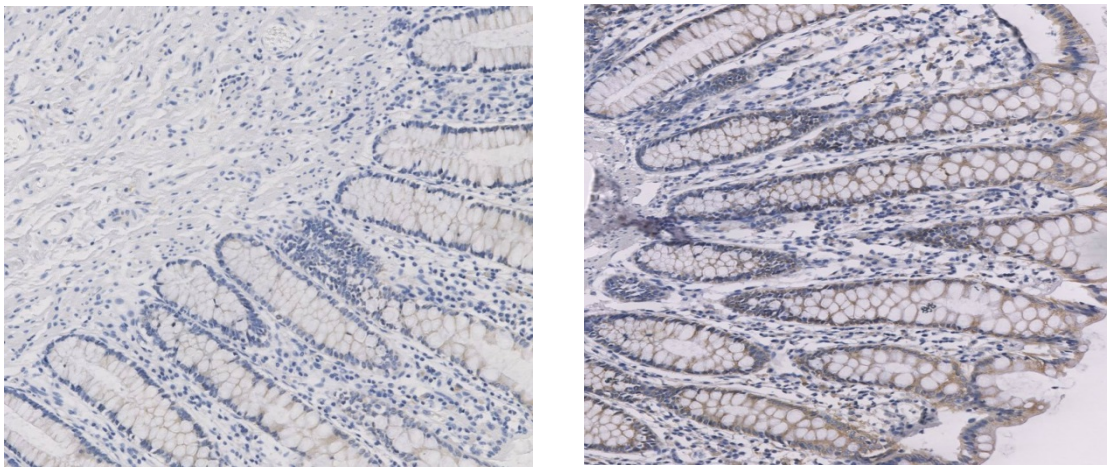


Figure 3-5: Human colon was used as a positive control for COX-2, MVD & Ki67. Pre-staining (left) & post staining (right)

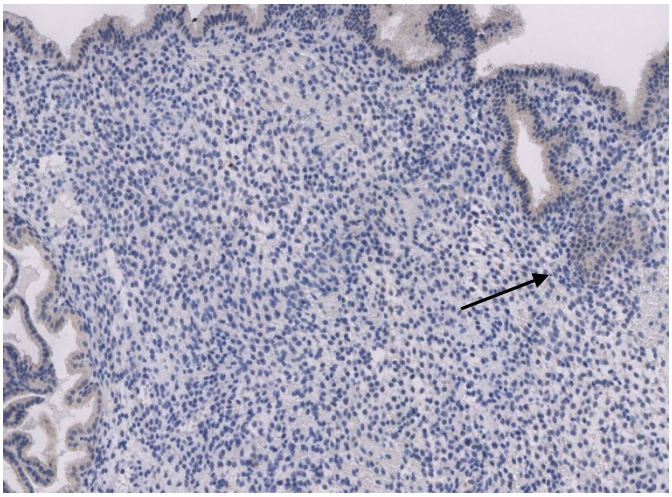


Figure 3-6: Weak expression of COX-2 in the endometrial stroma (arrow)

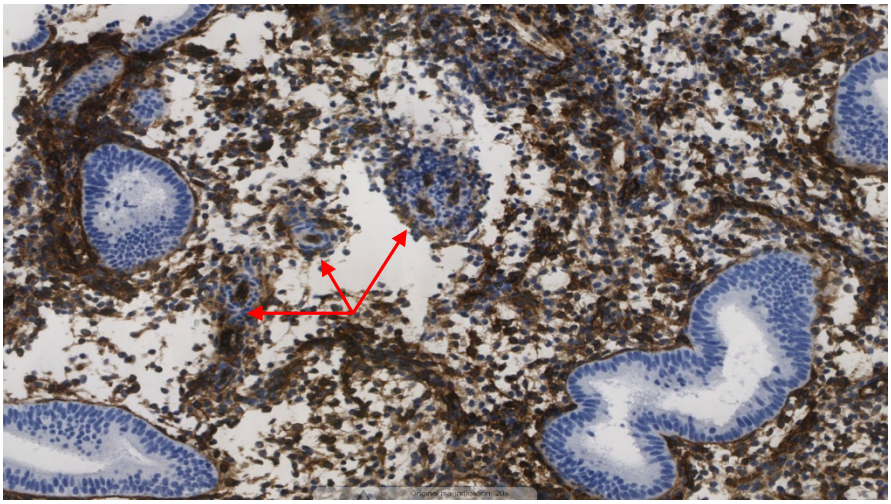


Figure 3-7: Micro vessels demonstrated after staining with anti-CD34

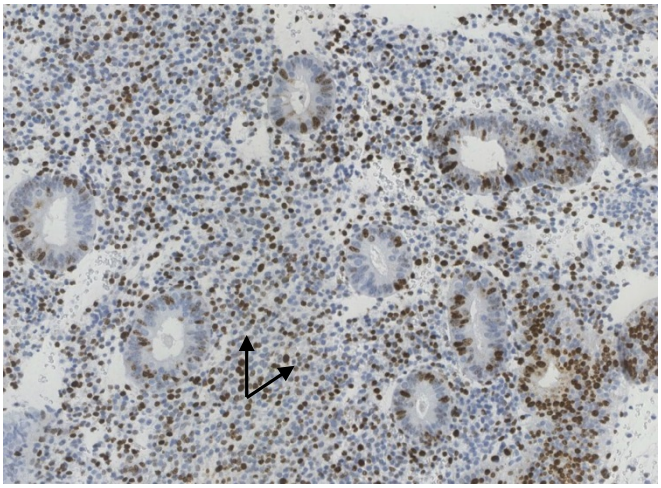


Figure 3-8: Moderate expression of Ki67 in the endometrial stroma (arrow)

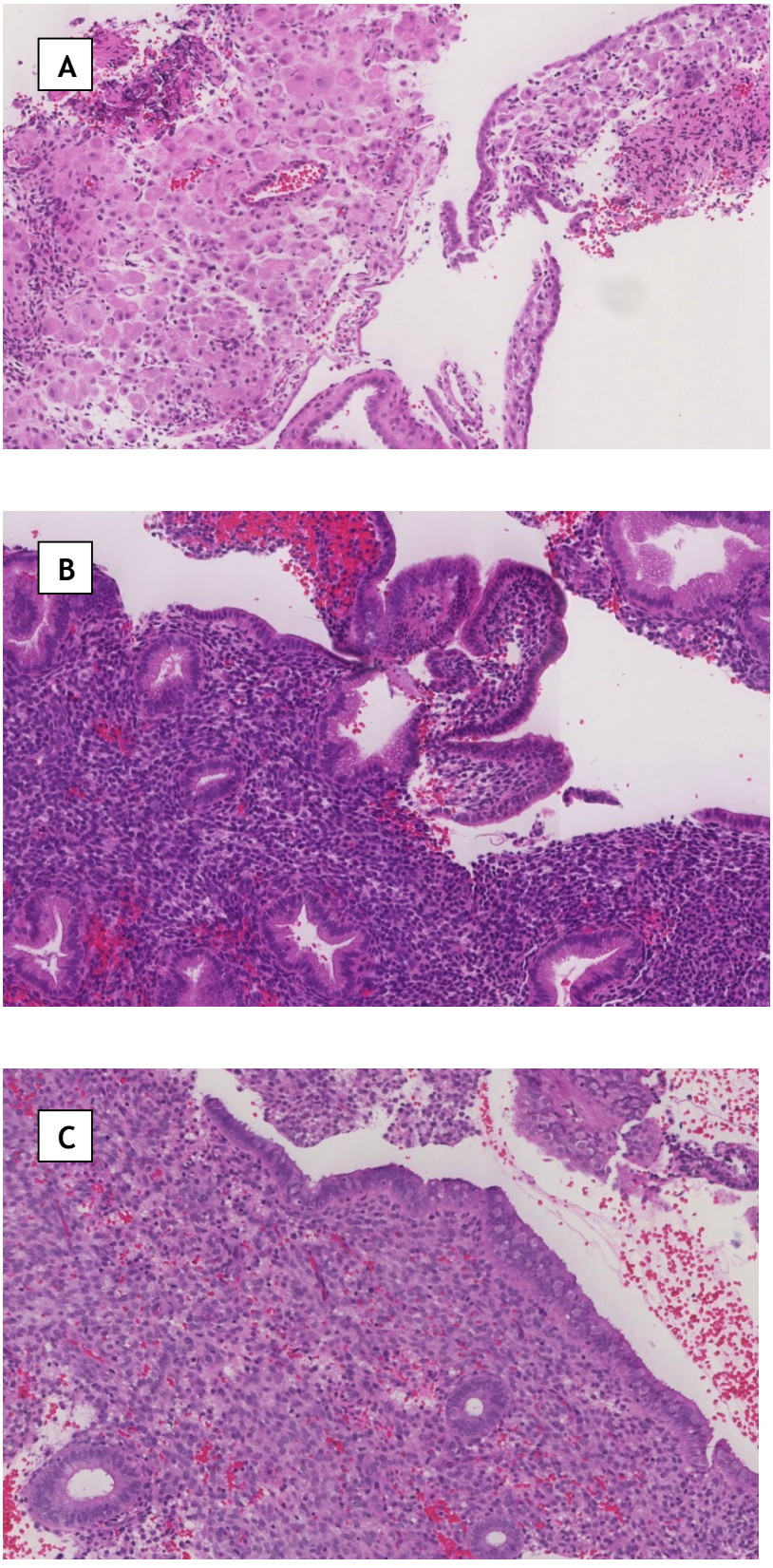


Figure 3-9: Stages of the endometrium as seen on H&E staining. A: Inactive, B: Secretory & C: Proliferative stage

3.5.5 **Quantitative RT-PCR for VEGF-A**

RNA was extracted with TRI-reagent (Ambion, life technologies, Paisley, UK) following manufacturer's guidelines using phase lock tubes (micro-centrifuge, Greiner bio-one GmbH, Stone House- England, UK). Using MultiScribe (Invetrogen, Life technologies, Paisley, UK) RNA samples were reverse transcribed. Then according to the manufacturer's instructions they were primed with random hexamers (see section 2.4). When no fresh endometrial sample was available, RNA was extracted from fixed paraffin embedded samples using Ambien kit 1975 (Recover All total nucleic acid isolation kit-Life technology, paisley, UK) according to the manufacturer's instructions (see section 2.4.1.1). Quality of RNA was assessed using an automated spectrophotometer RNA 6000 Nanodrop (Lab tech.com, Ringmer, East Sussex, UK), only half of the RNA samples were tested by the Agilent 2100 Bioanalyser system in combination with RNA 6000 nano chips (see details in section2.4.3). Once the RNA was extracted and quantified, the PCR reactions were carried out using an ABI Prism 7900 (Applied Biosystems) using duplicate samples. A no template control (containing water) was included.

The primer utilised in this study did not distinguish between the different VEGF isoforms. Pre-validated primers and probes were purchased for VEGF-A (Life technology, Paisley, UK). Gene expression was normalised to B-actin ribosomal RNA (Applied Biosystems) as an internal standard. Data were analysed and processed using Sequence Detector System (SDS version 2.3) according to the manufacturer's instructions. Results are expressed as relative to a standard cDNA obtained from a single sample of endometrial tissue and included in all reactions. The quantitative RT-PCR laboratory work excluding the statistical analysis was performed by researcher Salha Abukhnjr.

3.5.6 **Histo-scoring & analysis**

Scoring of immunostaining intensity of VEGF-A & COX-2 in stromal endometrial tissue was performed using the histo-scoring method.
The immunostaining of intensity of epitopes in VEGF and COX-2 antibodies stained sections was assessed in the semi-quantitative manner on the 4- point scale: 0= no immunostaining, 1= mild immunostaining, 2= moderate immunostaining, 3= intense immunostaining.
Training in histo-scoring was undertaken using the computerised image analysis system (Version 4.0, Digital Image Hub, Leica biosystems) as a direct feedback method. Once a strong correlation between scores derived from the image analysis and subjective scores by the observers was obtained, formal scoring was started. All tissue sections were scored both by me and one independent observer who was unaware of the patients' data. A histo-score correlation coefficient between the two observers was accepted at ≥ 0.7. Three separate areas that subjectively best represented each histology slide were scored at 10X and the average score was used in the analysis.

The computerised image analysis system (Version 4.0, Digital Image Hub, Leica biosystems) was used to assess proliferation through Ki67 expression. The stained cells measuring algorithm was optimised and analysis was performed at 20X. Three 3 separate areas that subjectively best represented the overall histology slide were analysed after annotating the areas to be scored using a polygon and avoiding glandular tissue. The average reading of the three automated cellular H-Score for nuclear staining was used as the overall Ki67 H-Score for that specific histology slide.

The automated image analysis system was also used in objectively measuring microvascular density (MVD). The micro-vessel detection algorithm was optimised with the help of Clare Orange. Slides that were stained with anti-CD34 were analysed at 20X. Although the automated system at times could not exclude larger vessels, it was thought that a standardised objective method of analysis for both before and after UAE would be superior to a subjective vessels calculation method. Density in a three dimensional structure is usually derived from mass divided by volume. However, we derived MVD by dividing the total

number of vessels by the area measured. The average view area for the computer monitor used was 230 μm. We noticed that the automated image analysis system used pixels as unit of length rather than μm (international system of units). Therefore we manually divided the number of vessels by 230μm to obtain a more accurate result rather than relying on the auto-calculation of the software.

3.6 Statistics

Data were subjected to statistical analysis using paired t-sample tests for normally distributed paired data. Some data that was non-parametric were normalised by natural logarithm prior to analysis. Mann-Whitney test was used to compare non-parametric non-paired data. Descriptive graphical analysis was used when there were too few samples to compare. Minitab 17 statistical software was used for the analysis and graphs were drawn using Microsoft Excel & Minitab 17.

3.7 Results

Endometrial sampling was undertaken in 18 patients both pre-UAE and 6 months post-UAE using the Pipelle® sampler. There were 6 samples that were inadequate for dating due to an insufficient endometrial sample being taken. Two samples from the same patient were only cervical tissue. Two post-UAE samples showed chronic endometritis were therefore excluded (table 2-1).

3.7.1 Paired and unpaired samples

In this chapter we will use the term "Paired samples" when both pre-UAE & post-UAE endometrial samples are from the same patient and same stage of the cycle (table 3-3). This will be used to form a direct comparison were the samples compared are from the same patient and at the same menstrual phase. Our aim was to achieve a high number of paired samples however this proved to be difficult.

The term "Non-paired samples" will be used when comparing endometrial samples from different stages of the cycle grouped together (table 3-4). This was used when the post-UAE sample was found to be at a different phase of the menstrual cycle. We felt there was still some value in performing such a comparison as it would show an overall change in the endometrium at different phases. Comparing non-paired samples also allowed us to use endometrial samples from patients that did not have an equivalent pre or post-UAE sample.

Table 3-4: Paired samples by menstrual cycle stage

Stage	Before UAE	After UAE
Proliferative	2	2
Early secretory	1	1
Mid secretory	2	2

Table 3-5: Total number of samples by menstrual cycle stage

Stage	Before UAE	After UAE
Proliferative	4	6
Secretory	6	4
Inactive/progesterone effect	3	3

3.7.2 **VEGF Expression:**

3.7.2.1 VEGF expression by immunohistochemistry

Analysis was first performed by comparing all pre-UAE samples to all post-UAE samples irrespective of the stage of the menstrual cycle or pairing based on advice and feedback from Professor Alistair Williams, Consultant Histopathologist, Edinburgh Royal Infirmary. We used a Mann Whitney U test and found no significant difference in the expression of VEGF between pre-UAE & post UAE samples (p=0.45) (Fig 3-10). The difference was still not significant when excluding inactive samples (p=0.73)

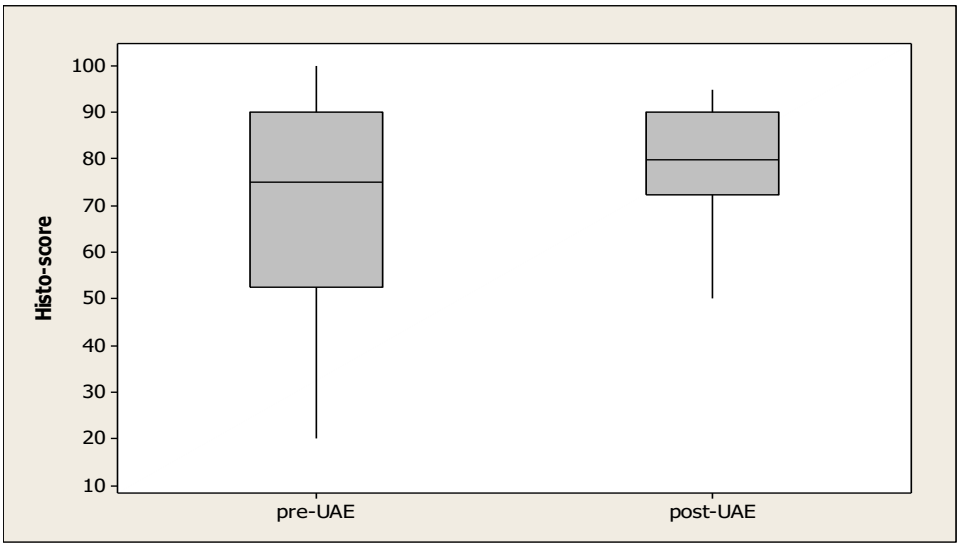


Figure 3-10: Box plot showing VEGF expression in all samples before and after UAE

We looked at all paired samples regardless of the stage of the cycle. As the samples were paired but not normally distributed we used the natural Log and analysed them using a paired t-test and found no significant difference in the H-Score pre & post-UAE (p=0.274) despite an observed upward trend (Fig 3-11).

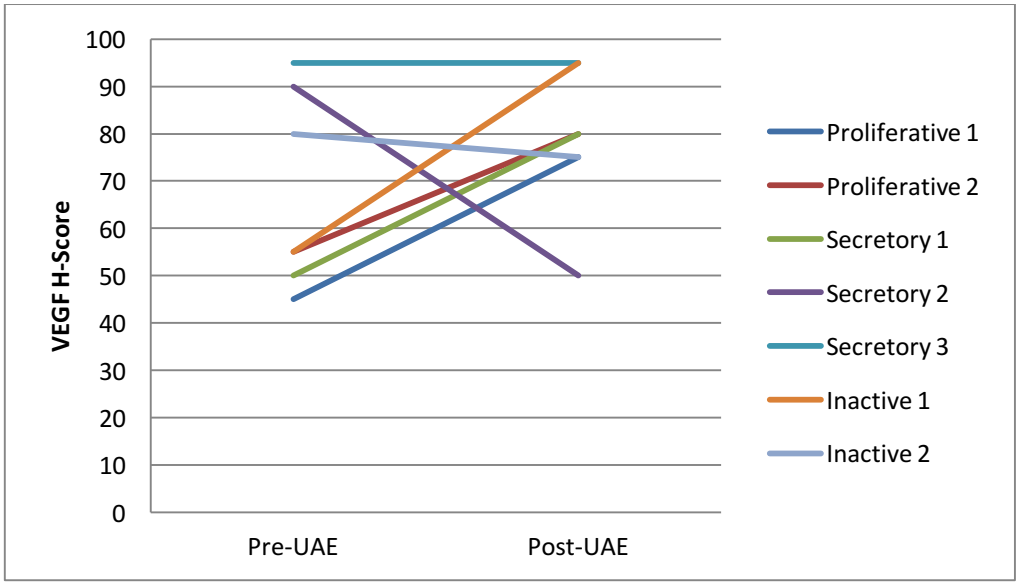


Figure 3-11: Line plot showing an upward trend of the expression of VEGF in the endometrium of patients with paired samples

However, when we looked at the paired samples in the proliferative group we found an increasing trend in VEGF expression post UAE. (Fig 3-12 & 3-13). A statistical test comparing two samples would not give an accurate result. This was not the case for the secretory phase or the inactive samples.

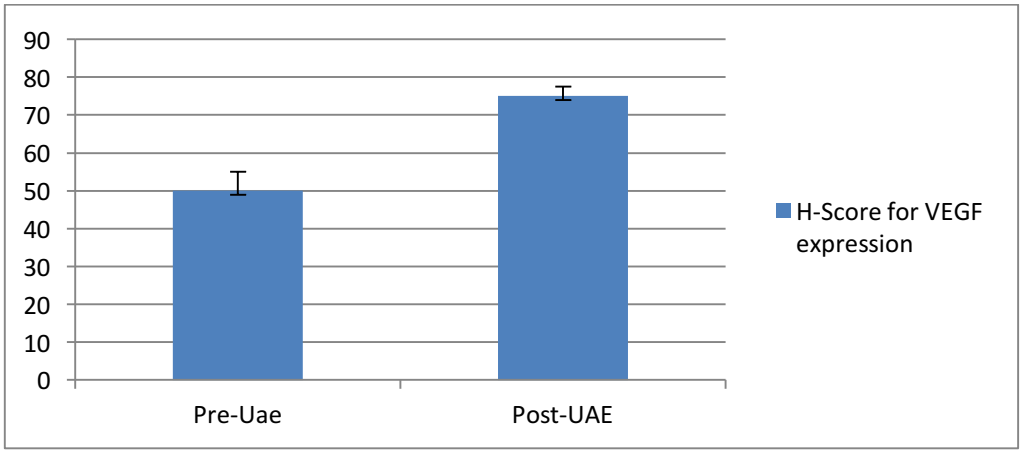


Figure 3-12: The change in VEGF expression in proliferative stage paired samples.

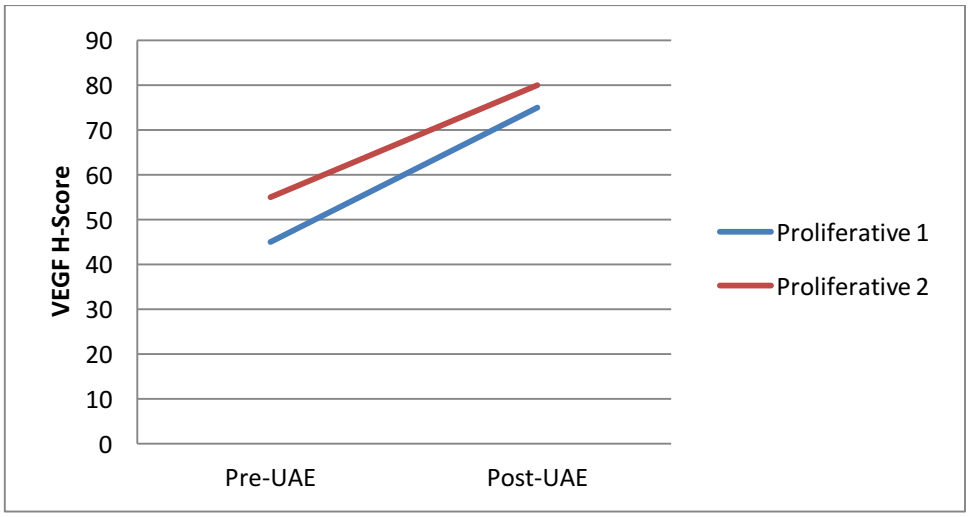


Figure 3-13: Line plot showing the change in expression of VEGF in the proliferative stage paired samples

Table 3-6: Summary of statistical testing for VEGF expression by immunohistochemistry

Group VEGF H-Score was calculated from	Pre-UAE N=	Post- UAE N=	Statistical test	Pre-UAE Mean ± STD or Median*	Post-UAE Mean ± STD or Median*	P=
All samples	13	13	Mann-Whitney	*75	*80	0.457
All samples (excluding inactive)	10	10	Mann-Whitney	*77.5	*80	0.73
Paired samples (all)	7	7	Log & paired T-testing	1.8 ± 0.13	1.89 ± 0.09	0.27
Paired samples (excluding inactive)	5	5	Log & paired T-testing	1.80 ± 0.15	1.88 ± 0.11	0.454

***Median for Mann Whitney U**

3.7.2.2 VEGF RNA relative expression by PCR:

Although the total number of paraffin block samples available for immunohistochemistry was 26 from 16 patients, we only had 10 frozen samples from 5 patients. As previously mentioned, when only a small amount of endometrium was obtained, we opted to send this to the histopathology department to be embedded in paraffin with the plan of future RNA extraction from paraffin (section 2.2.1). VEGF RNA relative expression was analysed from both frozen and paraffin block samples (Fig 3-15 & 3-16). A summary of the statistical analysis is included in table 3-7.

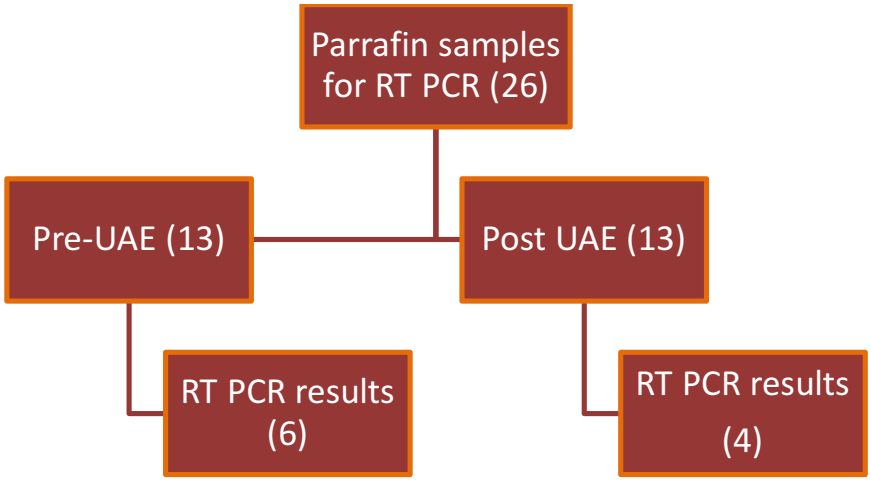


Figure 3-14: RT PCR for VEGF from paraffin blocks

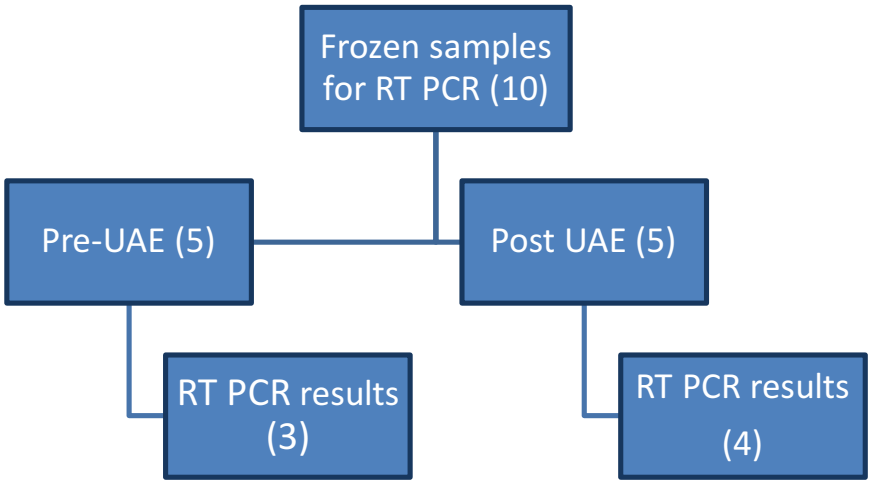


Figure 3-15: RT PCR for VEGF from frozen samples

3.7.2.2.1 Correlation testing

We looked at the correlation between the results extracted from paraffin to those extracted from frozen samples and we did not find any correlation. This may be due to the limited number of samples to compare.
We therefore analysed them as one group (paraffin & frozen) rather than two separate groups. We also looked at the correlation of the expression of VEGF by immunohistochemistry and that by RT-PCR (paraffin & frozen) and found no correlation between the results.

3.7.2.2.2 Comparing VEGF RT-PCR results extracted from both paraffin and frozen samples together

We compared pre-UAE VEGF RNA extracted from both frozen and paraffin block samples grouped together (n=9) to that post-UAE (n=8). As these samples were non-paired we used Mann-Whitney test and the results showed no significant difference (p=0.09) despite an upward trend in the samples post-UAE.

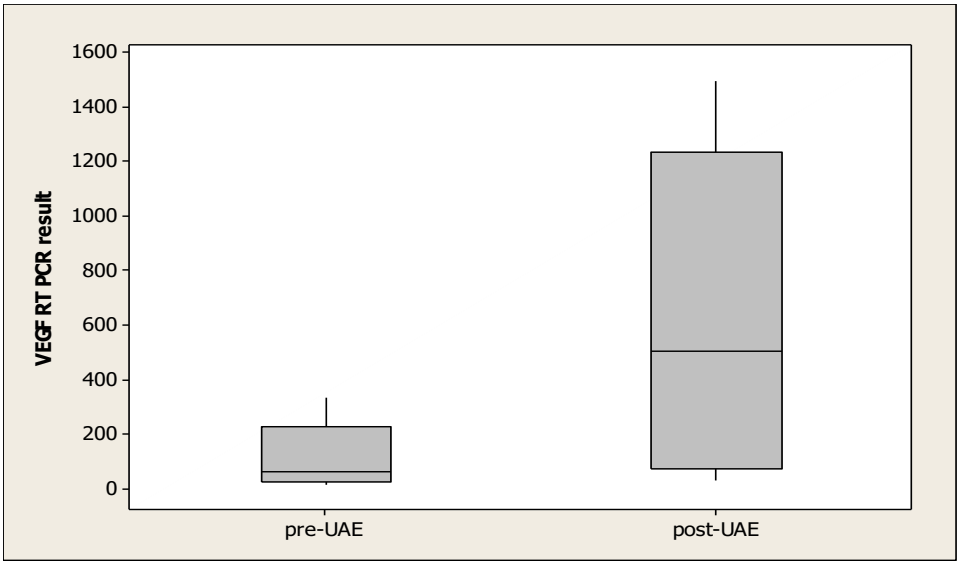


Figure 3-16 Box plot showing the difference in VEGF RT PCR pre & post-UAE extracted from both paraffin and frozen non paired samples grouped together

We then looked at only paired results from both paraffin and frozen samples. We used the natural logarithm as the results were paired but not normally distributed and tested with paired t-test. The results showed a significant increase in VEGF relative expression post -UAE (p=0.003).

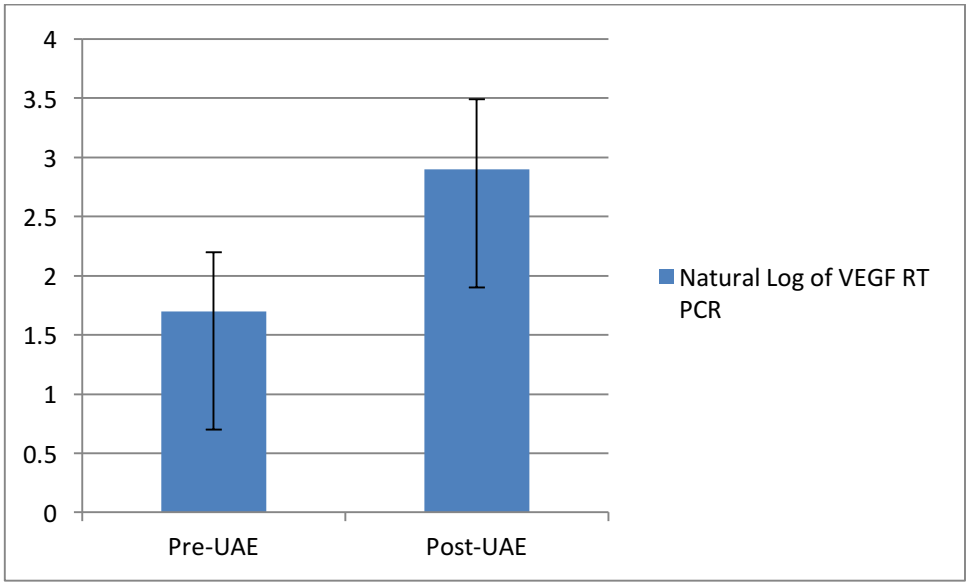


Figure 3-17 Bar chart showing the difference in the mean of VEGF RT PCR extracted from paired paraffin and frozen paired samples

3.7.2.2.3 Comparing VEGF RT-PCR results extracted from paraffin samples only

When looking at this group irrespective of stage of the cycle we found a significant increase in VEGF relative expression in post UAE samples (P= 0.036). We could not test the difference of paired samples as there was only one paired sample in the proliferative stage which showed an increase in VEGF relative expression in post UAE.

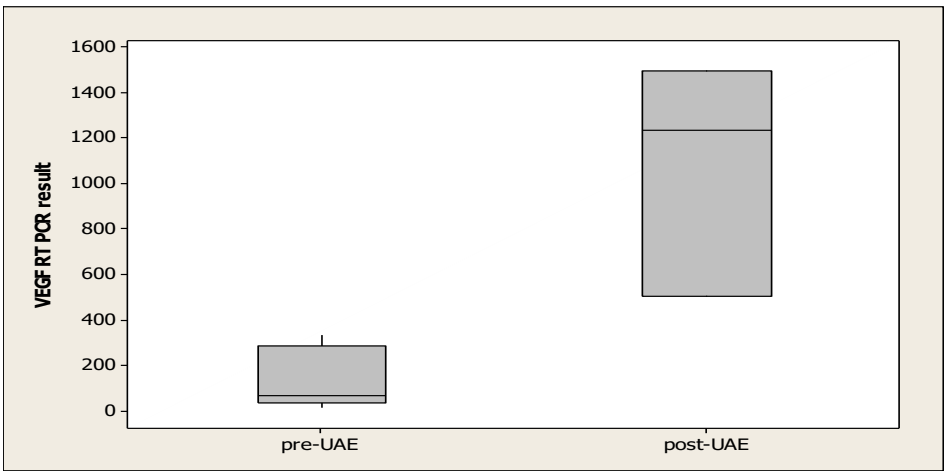


Figure: 3-18 Box plot showing the difference in VEGF RT PCR pre & post-UAE extracted from paraffin non paired samples only

3.7.2.2.4 Comparing VEGF RT-PCR results extracted from frozen samples only

We also looked at the paired results in this group irrespective of stage of the cycle (n=3). We used the natural logarithm as the results were paired but not normally distributed and tested with paired t-test. The results showed a significant increase in VEGF relative expression post-UAE (p=0.026).

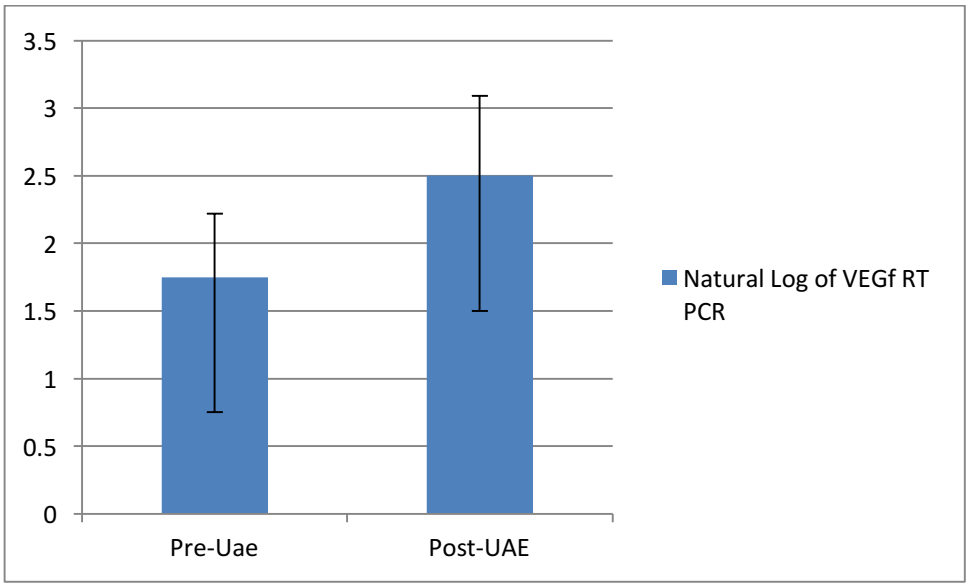


Figure: 3-19 bar chart showing the difference in the mean between VEGF RT PCR extracted from frozen paired samples

Table 3-7: Summary of statistical analysis for VEGF RT-PCR results

Group of samples VEGF was extracted from for RT PCR	Pre-UAE N=	Post- UAE N=	Statistical test	Pre-UAE Mean ± STD or Median *	Post-UAE Mean ± STD or Median *	P=
Paraffin and frozen (non-paired)	9	8	Mann-Whitney	*56.8	*425.7	0.09
Paraffin and frozen (Paired & excluding inactive samples)	4	4	Log & paired T-testing	1.9 ± 0.5	2.66 ± 0.59	0.003
Paraffin only (non-paired excluding inactive samples)	5	3	Mann-Whitney	68.5	1235.8	0.036
Frozen samples (paired results)	3	3	Log & paired T-testing	1.74 ± 0.47	2.49 ± 0.59	0.026

*** Median for Mann Whitney U test**

3.7.3 **COX-2 expression**

Analysis was performed by comparing all pre-UAE samples to all post-UAE samples irrespective of the stage of the menstrual cycle or pairing. We performed a Mann Whitney U test and found no significant difference in the expression of COX-2 between pre-UAE & post UAE samples (p=0.25). We also found no difference in COX-2 when we excluded all inactive sample results (0.07) (Fig 3-21).

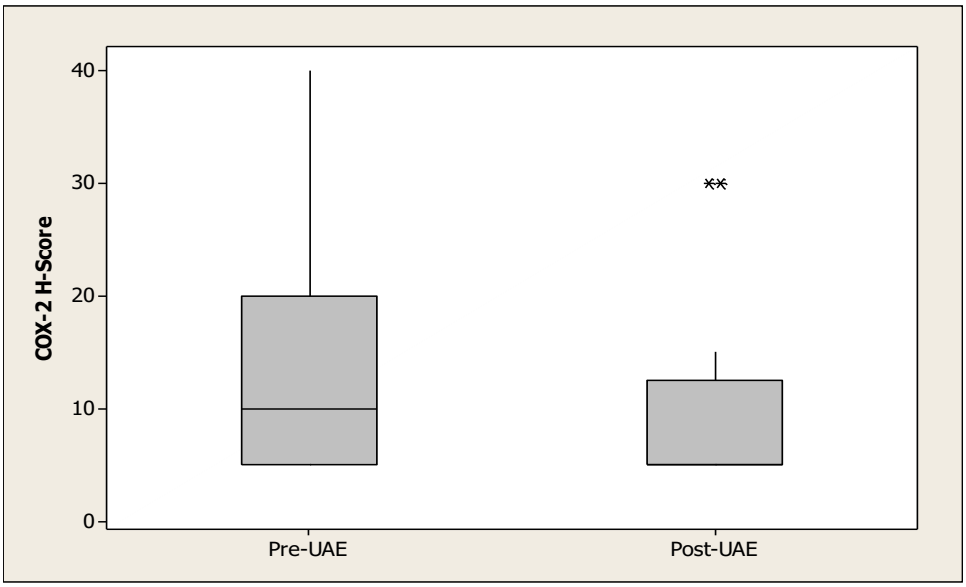


Figure 3-20: Box plot showing the change in COX-2 expression in all samples

We then looked at all paired samples regardless of the stage of the cycle. As these samples were paired but not normally distributed we used the natural Log and analysed them using a paired t-test. We found no significant difference in the H-Score pre & post-UAE (p=0.8). We also found no significant difference when excluding inactive samples (p= 0.07) despite an observed downward trend in COX-2 expression post-UAE (Fig 3-22.

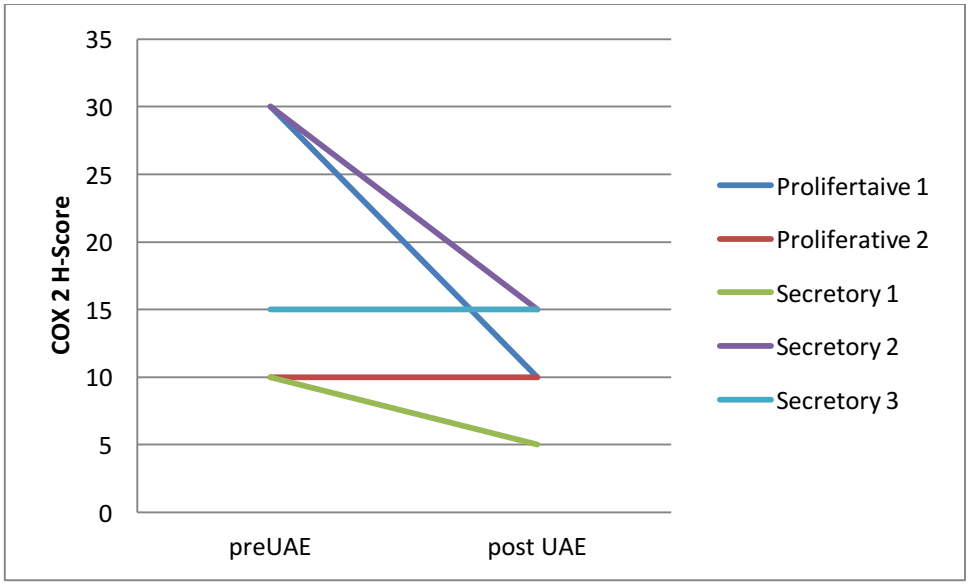


Figure 3-21: Line plot showing a downward trend of the expression of COX-2 in the endometrium of patients with paired samples (excluding inactive samples)

We then looked at the paired samples separately in the proliferative group, the secretory group & the inactive sample. Despite finding a downward trend we could not statistically test this due to the small number of paired samples.

Table 3-8: Summary of statistical testing for COX-2 expression by immunohistochemistry

Group of samples COX 2 H-Score was calculated from	Pre-UAE N=	Post- UAE N=	Statistical test	Pre-UAE Mean ± STD or Median*	Post-UAE Mean ± STD or Median*	P=
All samples	13	13	Mann-Whitney	*10	*5	0.25
All samples excluding inactive	10	10	Mann-Whitney	*10	*5	0.07
Paired samples (all stages)	7	7	Log & paired T-testing	1.05 ± 0.32	1.07 ± 0.32	0.8
Paired samples (excluding inactive)	5	5	Log & paired T-testing	1.19 ± 0.26	1.07 ± 0.28	0.51

***Median for Mann Whitney U test**

3.7.1 **Micro-Vascular Density**

The method used in measuring MVD has been explained in section 3.5.5. To clarify the values of MVD & to avoid negative LOG results all values in this section have been displayed as E-05 ($x10^{-5}$).
Analysis was first performed by comparing all pre-UAE samples to all post-UAE samples irrespective of the stage of the menstrual cycle or pairing. There was an observed increase in MVD post UAE. However, we performed a Mann Whitney U test and found no significant difference in the MVD of the endometrial samples pre-UAE & & post UAE (p=0.45) (Fig 3-22). We also found no difference when we excluded all inactive sample results (p=0.27).

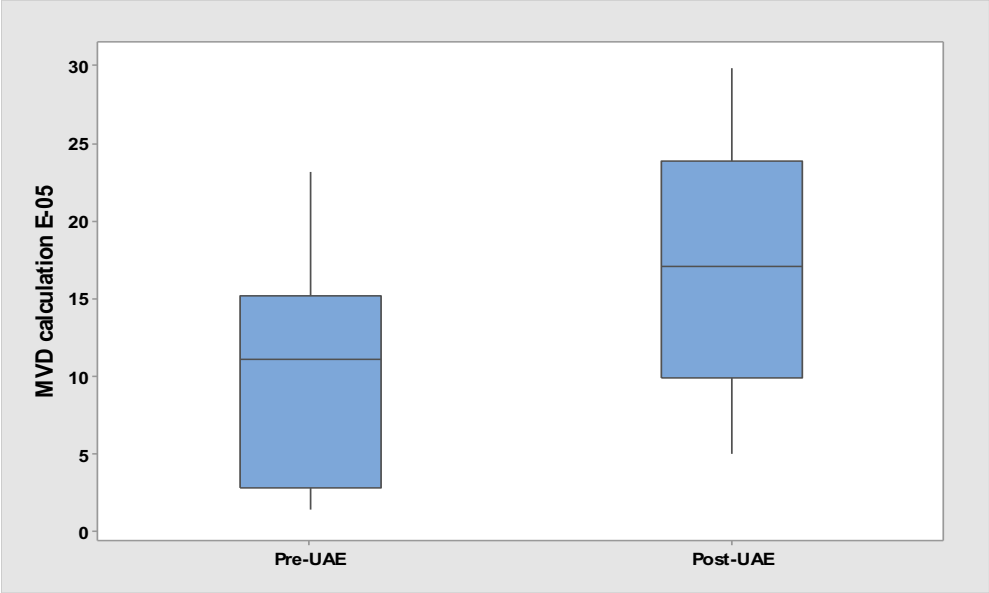


Figure 3-22: Box plot showing the change in MVD in the endometrium of all samples

We then looked at all paired samples regardless of the stage of the cycle. As these samples were paired but not normally distributed we used the natural Log and analysed them using a paired t-test. We found a significant increase in the MVD of the endometrium post-UAE (p=0.01) (Fig 3-23). Interestingly in samples from normally cycling women an observed increase in MVD post-UAE remained although the difference was not significant (p=0.1) (Fig 3-24).

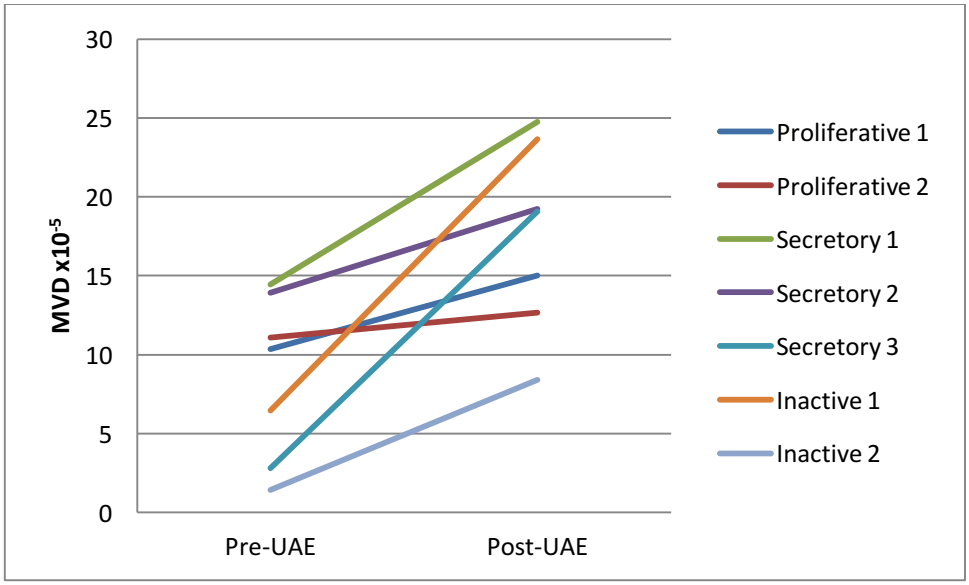


Figure 3-23: Line plot showing the change in MVD post-UAE for all paired endometrial samples

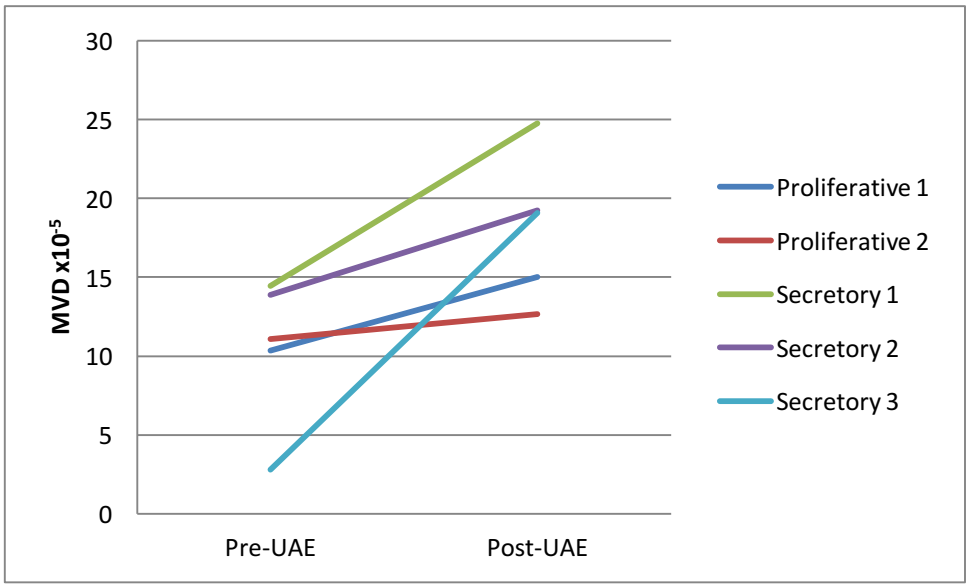


Figure 3-24: Line plot showing the change in MVD post-UAE for paired endometrial samples for cycling women

We then looked at the paired samples separately in the proliferative group, the secretory group & the inactive sample. Despite finding an upward trend we could not statistically test this due to the small number of paired samples.

Table 3-9: Summary of statistical testing for MVD by immunohistochemistry

Group of samples MVD calculated from	Pre-UAE N=	Post- UAE N=	Statistical test	Pre-UAE Mean ± STD or Median*	Post-UAE Mean ± STD or Median*	P=
All samples	13	13	Mann-Whitney	* 11.08 $x10^{-5}$	17.04 $x10^{-5}$	0.45
All samples excluding inactive	10	10	Mann-Whitney	* 14.13 $x10^{-5}$	16.02 $x10^{-5}$	0.27
Paired samples (all stages)	7	7	Log & paired T-testing	0.82 ± 0.38	1.29 ± 0.16	0.01
Paired samples (excluding inactive)	5	5	Log & paired T-testing	0.96 ± 0.29	1.24 ± 0.11	0.1

***Median for Mann Whitney U test**

3.7.2 **Ki67 expression (Proliferation marker)**

Automated scoring was used rather than manual scoring as Ki67 marker has been extensively used by Glasgow University pathology department previously. The automated scoring algorithm had previously been optimised and calibrated and required minimal change. Therefore no manual scoring was undertaken in this section.

All inactive samples were excluded in the analysis as they showed no proliferation. We first performed an analysis comparing all pre-UAE samples to all post-UAE samples irrespective of the stage of the menstrual cycle or pairing. We found the difference to be statistically insignificant (p= 0.23)(Figure 3-25).

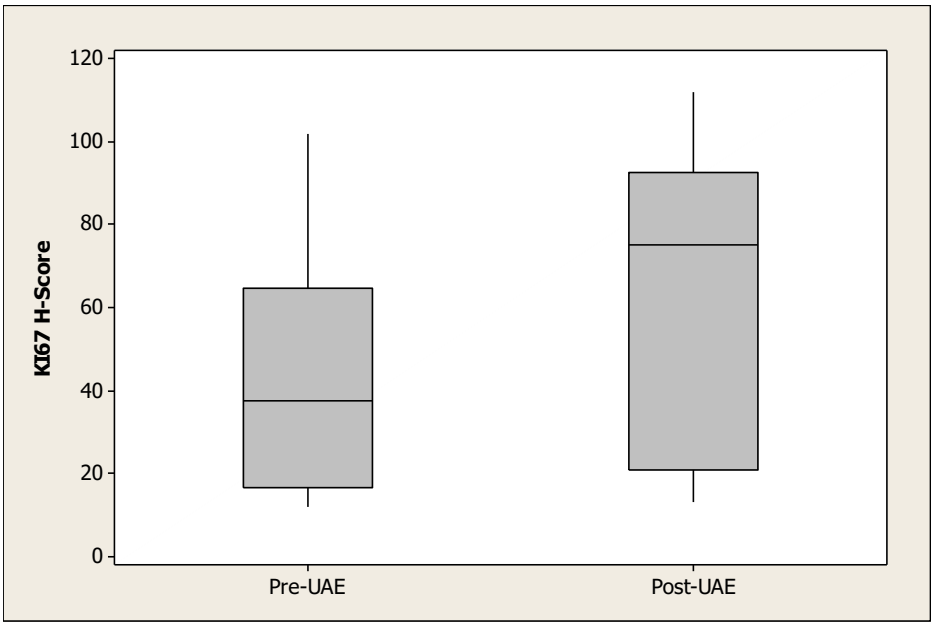


Figure 3-25: Box plot showing the change in Ki67 expression in the endometrium of all samples

We then looked at all paired samples regardless of the stage of the cycle. As these samples were paired but not normally distributed we used the natural Log and analysed them using a paired t-test. We found no difference in Ki67 expression post UAE for all samples (p=0.20).

We were unable to test paired samples according to the stage of the sample as the numbers were small. However we found an increasing trend in proliferation in proliferative stage samples post UAE. We looked at the secretory phase samples and it was only the early secretory phase sample had a similar trend (Fig 3-26).

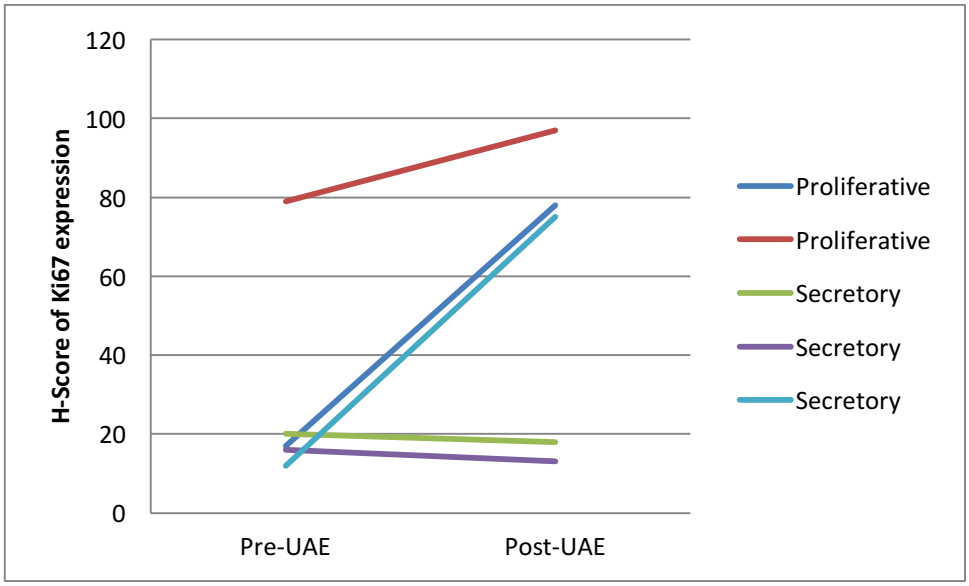


Figure 3-26: Line plot showing the change in Ki67 expression post-UAE for all paired endometrial samples

Table 3-10: Summary of statistical testing for Ki67 expression by immunohistochemistry

Group of samples Ki67 expression from	Pre-UAE N=	Post- UAE N=	Statistical test	Pre-UAE Mean ± STD or Median*	Post-UAE Mean ± STD or Median*	P=
All samples excluding inactive	10	9	Mann-Whitney	*37.5	*75	0.23
Paired samples (excluding inactive)	5	5	Log & paired T-testing	1.34 ± 0.32	1.62 ± 0.4	0.20

***Median for Mann Whitney U test**

3.7.3 **Discussion**

In this chapter we set out to find how UAE affects the endometrium and leads to cessation of heavy menstrual bleeding in women with fibroids. We looked at the expression of VEGF, COX-2, Ki67 and the microvascular density of the endometrium both before and after UAE.

We found an overall trend of increased VEGF expression and increased microvascular density in the endometrium post-UAE. We also found an overall trend of decreased expression of COX-2 post UAE. However, proliferation of the endometrium in the proliferative stage and early secretory stages of the endometrium seemed to increase post UAE. We could not assess statistical significance in many of the groups that were compared due to low sample numbers and this was the main weakness in this study.

We only looked at the endometrium in this study rather than fibroid tissue itself. To directly compare fibroid tissues would have required invasive interventional radiology biopsy sampling that is outside the scope of this small study. Endometrium was obtained through Pipelle™ endometrial sampling which is a relatively well tolerated procedure. A diagnostic dilatation and curettage (D&C) would have resulted in more endometrial tissue and sampled a larger aspect of the endometrial cavity [217]. It would have also avoided cervical samples that lead to a decrease in sample size. However, this would have required a formal general anaesthetic that would have been unethical to perform in an asymptomatic patient post-UAE. Also, we could realistically only obtain samples pre-UAE and once post UAE. It would have been beneficial to obtain samples immediately after UAE & also at 3 months post UAE however this would not have been tolerated by the subjects in the study and would have led to a high dropout rate.

Endometrial tissue that was collected was processed by the main pathology laboratory at the Queen Elizabeth Hospital and there is a standardised protocol for this. However, fresh samples that were frozen may have been affected by the two different methods of freezing after transfer from RNA Later. This was explained in the methodology in chapter 2. RNA extraction was successful in 10 out of 26 paraffin block samples. However 7 out of 10 frozen samples produced a RNA relative expression result. As there was only a small amount of endometrium frozen, RT PCR was only performed for VEGF.

We did however manage to look at four different markers in the endometrium despite the small amount of tissue obtained. The results are interesting and do point towards a change in these markers post-UAE and may well direct future research towards looking at other relative markers. The samples stored in paraffin have been added to the tissue bank and future sample collection may result in a better powered study that statistically significant results can be extracted from. The cost of this study was low as both pre & post-UAE MRI was part of the clinical assessment package for each patient. The researcher (myself) was a senior gynaecology registrar who is experienced in performing endometrial sampling and did not require training or supervision. Access to ward 56A at Princess Royal Maternity Unit and to Clinic F at Stobhill hospital was relatively easy for both the researcher and the subjects studied.

Immunohistochemistry is a well-established laboratory procedure and expertise in staining of some of the markers studied was available. The use of the IHC auto-stainer helped the researcher in maintaining the same standard for the different batches that were stained therefore reducing intra-observer variation.

We took all post-UAE samples at the time of the period that corresponded to the pre-UAE samples dating. Our aim was to pair these samples so that sub-group comparison could be made. Unfortunately, only 7/13 samples were paired and 2/7 paired samples were inactive and could not be dated to a specific stage of the menstrual cycle. The irregularity in the bleeding pattern post UAE was the main contributing factor in addition to subjects not able to attend an appointment for endometrial sampling at the required time.

Initial results were discussed with Professor Alistair Williams, Consultant Histopathologist, Edinburgh Royal Infirmary who advised that a meaningful result may be obtained by grouping samples from different stages of the cycle and looking at the overall difference. This type of whole group analysis was performed as well as sub-group analysis. However, the number of samples were small and at times we could only compare samples from two patients (n=2) and these results should be treated with caution. They do however open a debate for further research into this area and further narrow our search for the elusive endometrial marker that changes after UAE. This marker may then help in developing targeted strategies through translational research to alleviate the effects of uterine fibroids.

We analysed paired and non-paired samples with both inclusion and exclusion of inactive samples but found no statistically significant difference in VEGF expression. We observed the largest increase in VEGF expression in the proliferative stage of the cycle. This is the stage of the cycle when the endometrium regenerates & neovascularisation occurs. It may be that higher VEGF levels lead to a lower rate of aberrant vessel formation. We know that aberrant angiogenesis underlies abnormal menstrual bleeding associated with uterine fibroids [187]. The rise of VEGF may reflect new vessel formation or an ongoing endometrial repair process post-UAE.

We also found a sharp increase in VEGF RT PCR post- UAE in proliferative stage paraffin samples but as there was only one paired sample we could not statistically test it. As a total cohort, we found significant increases in VEGF RT PCR post UAE. This was true for paired paraffin & frozen samples grouped together excluding inactive samples, non-paired paraffin samples and non-paired frozen samples ($p= 0.003$, 0.036 & 0.026 respectively).

The COX-2 expression in the endometrial samples studied was mostly weak. It is usually at its highest while menstruating and no samples from this stage were obtained in our study [218]. When looking at the expression of COX-2 in the endometrium we observed an overall downward trend post-UAE which did not reach statistical significance. *Ma et al* described inhibition of VEGF expression after targeted interruption of COX-2 gene pointing to a directly proportionate relationship [219]. However, it may be that UAE has a global effect on endometrial marker, causing the observed inversely proportionate relationship between VEGF and COX-2.

We found an overall increasing trend in microvascular density post-UAE compared to pre-UAE in all paired samples. Such comparison yielded a significant result ($p= 0.01$). This is probably because the uterus as a whole has undergone an acute ischemic event and has compensated by forming new blood vessels. The statistical significance of these results was reduced after removing inactive samples most likely due to a decrease in sample size.

Despite a significant increase in VEGF & MVD in the post-UAE samples we did not find such an increase in the expression of proliferation marker Ki67. Under normal hormonal conditions, proliferation of the endometrium increases in association with VEGF & MVD and supports the thickening of the endometrium preparing it for implantation. Although there

was an increasing trend in Ki67 expression in the proliferative endometrium, there was no significant difference in the H-Score of paired samples (0.34).

Overall, we observed a change in our studied markers. There was an increasing trend in VEGF, Ki67 & MVD and a decreasing trend in COX-2 post UAE. The lack of statistical significance is likely to be due to small numbers. Future work that involves collecting further samples or re-testing for other markers is being considered.

4. Chapter 4: The factors that play a role in the success of UAE and its complications.

4.1 Introduction:

Uterine artery embolisation (UAE) is a non-invasive treatment for uterine fibroids. In 1995 *Ravina et al* was first to report that embolisation of the uterine arteries in a fibroid uterus would lead to avascular necrosis of the fibroid, causing volume reduction in the fibroid uterus and fibroids [132]. It has therefore emerged as a satisfactory alternative to hysterectomy and multiple myomectomies [148, 220].
There is widespread acceptance amongst gynaecologists and interventional radiologists of UAE as a treatment for uterine fibroids. UAE requires great familiarity with the pelvic arterial anatomy and variations in the vascularisation of uterine fibroid tumours may account for treatment failures. The uterine arteries which are branches of the anterior division of the internal iliac artery provide the predominant vascularisation for the uterus and the fibroid tumour [221]. However the ovarian arteries and the round ligament arteries also play a role in supplying blood to the uterus [222]. Angiography using pre-UAE MRI and intra-operative fluoroscopy is useful for comprehensive assessment of the anatomy of the internal iliac artery, its pattern of division, and its branches.
In many reports, heavy menstrual bleeding associated with fibroids is improved in 85% of patients who undergo UAE [220]. The likely explanation is due to reduced blood flow and changes in vascular supply to the fibroid uterus. Pressure symptoms are also helped in 70-90% of patients, this is mainly due to uterine volume and fibroid volume reduction of up to 40% [223].

Imaging is very important in pre-procedural planning for uterine fibroid embolisation. Deciding who is an appropriate candidate for the procedure involves careful evaluation of uterine fibroid tumours, particularly their number, size, and location and the degree of vascularisation which can be achieved using different imaging modalities. Magnetic Resonance Imaging (MRI) facilitates accurate imaging and assessment of symptomatic uterine fibroids and helps in evaluating the efficacy of UAE. Pre-embolisation MRI is recommended by many radiologists because it allows differential diagnosis of other diseases that can mimic fibroids such as adenomyosis and other space occupying pelvic lesions such as solid ovarian cysts [224]. Fibroids usually appear isointense to the surrounding myometrium on T1 weighted imaging (T1-WI) and hypointense on T2 weighted imaging (T2-WI).

The current standard of practice is to use a gadolinium-based contrast agent (GBCA) enhanced pelvic MRI. When gadolinium is used as a contrast agent, then fibroids that have a vascular supply appear to enhance [225]. This then helps monitor the response to treatment by subjectively comparing before and after treatment contrast enhancement. Response is also assessed by the measuring the change in both volume and vascularity of fibroids post UAE.

The morphology of a fibroid can change and can be described as necrotic, calcified or oedematous. The MRI signal can change once treatment is initiated. After UAE, early MRI signals show disappearance of contrast enhancement and subsequently a volume reduction of the fibroids. These signal characteristics are secondary to hyaline degeneration of the fibroid [224]. They are considered as morphological imaging criteria that are in keeping with a successful procedure [226]. However, this may not always reflect resolution of clinical symptoms but may aid the clinician and the patient in planning further treatment.

4.2 The standard pelvic MRI analysis:

The pelvic MRI that is obtained in a woman with a suspected fibroid is usually reported/analysed by a consultant radiologist with a special interest in gynaecology MRI. It

may also be reported by a middle grade doctor in radiology (registrar) under direct supervision of a consultant radiologist. The images produced by the MRI scanner can be rendered by the MRI software to scan through the subject imaged in both coronal and sagittal planes producing a three dimensional image.

The factors taken into consideration when viewing an MRI of a fibroid uterus to assess the suitability for UAE are:

4.2.1 **Number of Fibroids**

Women present with one or more fibroids within their uterus. Although the number of fibroids can help assess suitability for surgery such as myomectomy, there is no evidence that they can influence the outcome.
Firouznia et al demonstrated an association between fibroid number pre-UAE and outcome measures post-UAE, including size reduction of the fibroid and the uterus. The reduction in mean fibroid volumes was similar in patients with single and multiple fibroids [227]. Another study failed to predict the clinical outcome after UAE of women whom presented with a single fibroid [228].

4.2.2 **Size of Uterus/Fibroids**

There have been a few studies looking at the size of fibroid uterus and its response to UAE. *Parthipun et al* found no increased incidence of complications in women with large-diameter fibroids (large fibroids were classified as ≥ 10 cm) and large uterine volumes (large uterine volume were classified as ≥ 750 cm^3)[229] . *Katsumori et al* also looked at uterine fibroids with any single diameter of 10cm or larger as a risk factor and no increased risk was found to patients undergoing UAE with such large fibroid size [230]. *Berczi et al* compared fibroids with <10 cm largest diameter to those >10 cm and found no significant difference in the effectiveness of UAE treatment or complication rate [231]. *Prollius et al* considered a uterine volume of ≥ 780cm^3 to be a large uterus rather than relying on a single measurement of >10cm. *Smeets et al* used a cut-off volume of 700cm^3 to differentiate between small and large fibroids and found no difference in the effectiveness of UAE between these two groups [232]. We will use similar cut-offs in the analysis later in this chapter.

Another study looked at the correlation between fibroid and uterine volume to the menstrual blood loss and found a negative correlation between the diameter of the largest fibroid to the menstrual blood loss and the same for uterine volume [73].
The importance of the size of a fibroid is still a matter of debate. There is no definite answer on whether UAE should be offered as a treatment modality in women with large fibroids.

4.2.3 **Location of fibroids**

Both pelvic MRI & pelvic ultrasound are used in determining the location of uterine fibroids, however MRI has been shown to be highly accurate in determining uterine fibroid location [225, 233]. The fibroid tumour location seems to play a more important role than fibroid size in governing the outcome of UAE role [234].
Submucosal fibroids exhibit volume reductions greater than 50% by magnetic resonance imaging after UAE [235]. However, women with submucosal leiomyomas at the time of embolisation are more likely to have post-procedural complications as these fibroids can migrate into the cavity after UAE. The majority of these are expelled spontaneously without significant symptoms. In some instances, submucosal fibroids greater than 6 cm in size that become endocavitary may cause complications requiring further intervention. The intervention is usually in the form of dilatation and curettage that is usually performed under a general anaesthetic. It is important to consider this when counselling patients contemplating UAE [236, 237]. Such fibroid expulsion is not limited to submucosal fibroids and there have been case reports of expulsion of intramural fibroids [238, 239]. Expulsion of myomas after uterine artery embolisation occurs relatively frequently and may be one of the ways to attain cure rather than be considered a complication.

4.2.4 **Degree of vascularisation**

In the initial studies about the outcome after UAE, ultrasound was used to evaluate the uterus and the fibroids after treatment [132]. The disappearance of fibroid vascularisation was observed using Doppler ultrasound after successful embolisation [240]. Although ultrasound is inexpensive, it does have limitations when assessing fibroids both pre & post UAE. Therefore, most current UAE procedures are followed up by contrast enhanced MRI

which shows signal intensity changes including increased signal intensity on T1-weighted images and homogeneous decreased signal intensity on T2-weighted images. MRI can also show lack of contrast uptake that is consistent with haemorrhagic infarction and hyaline degeneration [241].

Contrast enhanced Magnetic Resonance Imaging (MRI) before UAE is highly useful in the evaluation of patients referred for UAE. Gadolinium based contrast enhanced MRI is used to determine the viability of tumours and detect other findings prior to UAE. A woman with non-viable fibroid tumours should therefore not undergo UAE [242]. The degree of infarction (lack of contrast enhancement)
of uterine fibroids after embolisation on enhanced MRI is related to long-term clinical outcomes. Complete infarction of fibroid tissue induces a higher rate of symptom control, compared with incomplete infarction of fibroid tissue. This is reflected by a lower rate of post-procedure intervention [243].

An infarcted fibroid that has responded well to UAE appears dark on contrast enhanced MRI. This reflects the absence of contrast material, which in itself reflects a lack of vascularity of the fibroid and therefore procedure success from an imaging aspect (fig 4-1).

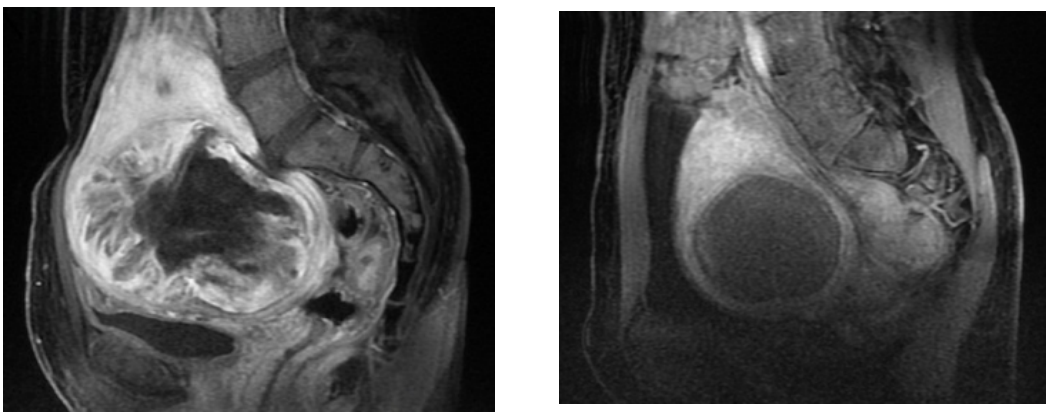


Figure 4-1: Fibroid showing contrast enhancement pre-UAE (left) and non-contrast enhancement post UAE (right).

4.2.5 **Age of Patient**

Uterine fibroids are more common in adults than in adolescent females. In one study the mean age of women who reported a diagnosis of uterine fibroids was 8 years older than women without the diagnosis [244]. There has been a report of a symptomatic fibroid in a girl as young as 13 years old [245]. Other case reports of girls as young as 16 years presenting with large fibroids and requiring surgery are in the literature [246, 247]. *Fields et al* has produced a case series of young-adult women between the ages of 13 and 21 and compared their management with the adult population [248]. *Van Voorhis et al* looked at risk factors for development of fibroids and found increasing age was nearly significant as a risk factor [249].

A few studies have looked at age as a predictor of response to UAE, these have found that age did not correlate with a reduction in the size of fibroids after UAE [250, 251]. However, *Jha et al* found that increased age was a predictor of failure [252].

The age of the patient when UAE is performed may become more important when comparing the long-term results from studies involving patients who are near the age of menopause, when there is a greater likelihood that the symptoms will resolve. This could be due to age-related changes that occur to the uterus in general and to the fibroid in particular in this age group rather than the effect of UAE alone [253]. It may be fair to say that some synergistic mechanism between age-related changes and UAE is more pronounced nearer to menopause.

4.3 MRI of the endometrium following UAE:

The endometrium demonstrates a wide spectrum of normal and pathologic appearances throughout menarche, during the pre-pubertal and postmenopausal stages of a woman's life. Imaging with ultrasound is almost always the first modality used to assess endometrial disease due to it being inexpensive, non-invasive and correlates well with MRI [254]. Although it is well documented that MRI is useful for assessing the quantitative & morphological changes of the fibroid uterus before and after UAE [252]. The endometrial changes observed on MRI following UAE have not been widely studied. There have been some reports of UAE-associated endometrial thinning that are linked to placenta accreta in a subsequent pregnancy [255]. Permanent amenorrhea secondary to endometrial atrophy

after UAE is a risk that patients should be counselled about. Such complication could lead to a devastating outcome for a women wanting to become pregnant as it may lead to reduced fertility after embolisation or the inability to become pregnant secondary to failure of implantation [256].
There have been studies that have used MRI to look at endometrial proliferation and thickening during the use of tamoxifen for breast cancer. Tamoxifen has pro-oestrogenic effects on the endometrium and can lead to hyperplasia and endometrial cancer. These studies have recommended MRI as a diagnostic tool in following up endometrial changes [257, 258]. MRI could therefore be a useful diagnostic tool in assessing endometrial changes post UAE.

4.4 The UAE procedure:

The main objective of UAE is to completely infarct the fibroid tissue within the uterus while preserving the uterus, ovaries and surrounding pelvis tissues. This is achieved by completely occluding the uterine arteries with an embolic agent using a percutaneous approach. Stasis of contrast material in the uterine arteries is the usual angiographic end point that signifies a technically successful UAE procedure. The procedure is concluded when a standing column of contrast material is observed in the uterine artery or when reflux of contrast material toward the uterine artery origin or into the hypogastric artery is observed under fluoroscopy [132, 220].
UAE is performed by an interventional radiologist who is competent in embolisation techniques. A C-arm fluoroscopic unit which supports road mapping of the vessels is used. In order to lower radiation exposure, the radiologist should use modern angiographic units with flat panel detectors and strictly apply dose reduction methods such as pulsed fluoroscopy [259].

The patient is usually consented in the outpatient department prior to the procedure or on the day of the procedure. This should include explaining the risk of failure of the procedure and the risk of developing uterine ischemia that can lead to an emergency hysterectomy and irreversible loss of fertility [260]. Ovarian failure can also complicate UAE due to uterine artery and ovarian artery anastomosis and has been found in 7% of the population [261]. In such cases coil embolisation may be required prior to UAE to prevent non-target embolisation of the ovary with embolic material particles [262, 263].
Although the majority of women undergoing UAE have heavy periods, amenorrhea is not usually what they desire especially in women undergoing UAE as a fertility-sparing option. Endometrial injury caused by ischemic endometrial damage is usually the cause of post procedural amenorrhea [263].

Patients are admitted to hospital on the day of the procedure to a gynaecological, surgical or vascular ward. Pain is expected as an early side-effect of successful UAE and joint protocols should be in place and adhered to in managing post UAE pain (Appendix 4). Vascular access with percutaneous femoral artery approach is followed by trans-catheter delivery of the embolic agent into the uterine artery on one side under fluoroscopic guidance. The embolic agents used are either gelfoam or polyvinyl alcohol embolisation particles (PVA). Once stasis is achieved, the contra-lateral uterine artery is then embolised in a similar fashion.
Once stasis of both uterine arteries has been achieved, the catheter is removed and haemostasis over the femoral artery is achieved by manually pressing over the incision area for up to 5 minutes to compress the femoral artery and prevent haematoma formation. The procedure time is reported to average around 61 min and fluoroscopy time (X-ray radiation exposure) around 19 min [223].

4.5 Complications after UAE

When comparing UAE to other forms of treatments for fibroids, re-intervention rates and long term follow up should be taken into account. The majority of patients undergoing UAE will be followed up for 6 months post procedure. This is usually in the form of imaging studies with contrast enhanced MRI being the most informative. However, the use of ultrasound for fibroid uterus volume assessment post UAE is also reliable [264].

There have been two main studies that have compared UAE to surgical intervention for the treatment of fibroids. These studies are the REST and EMMY trials. Both being randomised controlled trials and both followed up the patients for a period of five years. Although the primary outcome of both these trials was to look at the quality of life of women after fibroid treatment. The secondary outcomes included re-intervention rates [148, 265].
The largest series to date that has assessed complications secondary to UAE is the FIBROID registry which involved 3000 patients. It reported around 5% major adverse events in the first 30 days following hospital discharge [266]. A broad range of complications can occur after UAE and the rates of these vary widely between studies. One study by *Spies et al* looked at 400 consecutive UAE procedures and reported a peri-procedural complication rate of 8.5% with a serious complications rate of 1.25% [267]. Later in this chapter we will be looking at re-intervention rates in a cohort of patients in Glasgow who underwent UAE. We will try and establish whether the initial fibroid size pre-UAE and fibroid shrinkage 6 months post-UAE correlate to re-intervention rates up to 18 months post UAE.

4.5.1 **Post embolisation syndrome (PES):**

It is a post procedural complication that is reported in up to 3% of women undergoing UAE but some estimate a higher incidence [268]. It is defined as the development of post embolisation pain associated with nausea and/or vomiting, generalised malaise, low grade fever and a mild to moderate rise in inflammatory markers [269]. It usually affects patients who have infarcted all or part of a bodily organ after an embolisation procedure and in UAE this organ is usually the uterus. The pain after UAE is usually pelvic in site and crampy in nature and usually starts 2-3 hours post embolisation. *Pron et al* looked at post UAE pain which rose from a pre-UAE score of 0/10 in the majority of patients to an average score of 7/10 post-procedure [223].
The cause of PES in UAE is thought to be ischemia of the normal myometrium [270]. The mechanism of ischemia is not fully understood and it has only been studied best in patients with cardiac-origin angina pain. This mechanism is thought to be by formation of adenosine triphosphate, which secondary to anaerobic metabolism leads to acidosis and the loss of cell membrane integrity. Intracellular chemical mediators such as lactate and adenosine are released which effect chemo-sensitive receptors and therefore cause PES [271].

4.5.2 **Infection:**

Uterine infection is a complication of UAE which may occur soon after UAE but can also occur weeks or months post procedure [267, 272]. It can present in the form of sudden onset severe pain, vaginal discharge and/or bleeding. Infection should be aggressively treated therefore; readmission to hospital post UAE is common. Patient education is vital to avoid late presentation and serious complications such as hysterectomy [273]. The incidence of complications secondary to intrauterine infection was reported as 1.2% in a case series of 414 UAE procedures. This required intravenous antibiotic therapy and/or surgery [274]. As the symptoms of infection and PES overlap it would be beneficial to treat PES with antibiotics and therefore avoid late complications such as sepsis.

4.5.3 **Fibroid expulsion and need for hysteroscopy:**

There is a risk of fibroid expulsion, when UAE is performed in the presence of a submucosal fibroid or an intramural fibroid with a substantial submucosal component. Although most women tolerate fibroid expulsion well, approximately 50% may need surgical intervention [275]. The expulsion of an infarcted fibroid often leads to further intervention in the form of hysteroscopy, transvaginal myomectomy or even hysterectomy [276]. However, *Laverage et al* reported a case of multiple large fibroids being expelled vaginally after UAE without the need for re-intervention [277]. Nulliparous women showed a trend toward undergoing urgent and elective hysterectomy to manage such complication compared with parous women [275]. This may be due to difficulty in dilating the cervical os. The use of PGE2 such as vaginal misoprostol prior to hysteroscopy in cases of fibroid expulsion may prove to be of benefit. This method is called cervical primary and has been used in elective trans-cervical fibroid resection and found that it reduced the need for cervical dilation and facilitated such surgery [278].

4.5.4 **Non-target embolisation (off-target organ embolisation):**

Ovarian vasculature embolisation due to utero-ovarian collaterals has been well documented. This leads to degradation of ovarian function, which increases the risk of

premature menopause especially in women over 45 years old [135]. One small study found that women under the age of 45 who underwent UAE experienced transient post-menopausal symptoms which included amenorrhea, hot flushes and elevated FSH. This was observed in up to 6% of women undergoing UAE and usually resolved by 10 months post UAE [279]. The loss of ovarian function can be very distressing for women wanting to preserve their fertility and appropriate counselling should be undertaken.
The uterine artery can sometimes share a common trunk with the vaginal arteries or forms an anastomosis within the broad ligament [280]. This can lead to embolic particles passing through the vaginal arteries resulting in vaginal ischemia. This can lead to sexual dysfunction and chronic dyspareunia [281] . One of the rare identified causes of non-target embolisation is due to intra-fibroid arterial-venous fistula which was reported as fatal [282]. Other forms of interventional radiology treatments use emboli coils to induce stasis and there have been case report regarding coil migration that may lead to catastrophic outcomes (appendix 5).

4.5.5 **Venous Thromboembolism (VTE)**

It is thought that VTE such as pulmonary embolism (PE) & deep venous thrombosis (DVT) develop after UAE due to development of a prothrombotic state similar to that seen in a surgical procedure [283]. There have been 2 case reports of PE after UAE which have been fatal [284, 285]. Luckily, the incidence of such events is low and has been reported as (0.3%) [286].
DVT reported as a complication after UAE has a reported incidence of 0.4-0.5% [267, 286]. It has been reported just days after the UAE procedure with the majority of cases occurring within 3-5 days post UAE.
There should be a high level of suspicion of VTE after UAE when a patients presents with symptoms suggesting PE or DVT. Patient education has an important role in early presentation and diagnosis.

4.5.6 **Re-intervention (UAE, myomectomy & hysterectomy)**

Initial long term data showed the risk of re-intervention following UAE to be 20% [287]. Re-intervention in this context is defined as a subsequent need for repeat UAE, myomectomy

or hysterectomy. Data published from the EMMY trial and the REST trial showed even higher re-intervention rates [148, 265]. In the EMMY trial follow up data where recorded for 81/88 patients that underwent UAE. This showed 23/81 (28.4%) subsequently had a hysterectomy due to lack of symptom improvement. In the REST trial 106 patients underwent UAE with a 5-year re-intervention rate of 32% for treatment failure or complications [148].
The high re-intervention rate may influence how widely UAE is offered in a financially struggling national health service (NHS).
Although cost is a factor when comparing UAE to hysterectomy, it may be that a hysterectomy is not an acceptable option for some patients. Inarguably fertility ceases with hysterectomy however, UAE aims to at least preserve it or even improve it. Currently there is an ongoing trial comparing the quality of life for women randomised to either myomectomy or UAE (FEMME trial). Data from this trial will also look at re-intervention rates in both these two groups up to 5 years after the initial procedure. In this chapter we will look at short term re-intervention rates following UAE.

4.5.7 **Death**

There have been reported cases of death following UAE. Fortunately, the mortality risk is low at 0.05 per 1000 which compares favourably to the mortality risk of hysterectomy ~0.38 per 1000 [288, 289]. Death has been reported mainly secondary to septicaemia and sepsis from a necrotic uterus [290, 291]. Cases of PE and non-target embolisation are other causes of death that we mentioned earlier.

4.6 Patients and methods:

4.6.1 Subjects:

The study population was identified using the Clinical Research Information System (CRIS) at Gartnavel Hospital, Glasgow. Patients who had undergone their first UAE procedure between 1st of January 2010 and 31st of December 2014 in NHS Greater Glasgow and Clyde (n=171) were identified using a search by exam name: Embolisation of uterine fibroid, code IUTFDE.

4.6.2 Exclusion criteria:

1-Subjects having a repeat UAE within the study period.
2-Subjects with incomplete MRI imaging.
3-Women who had the post UAE MRI not within the follow-up period for this study.
4-Women who had two UAE procedures in the study period, the second UAE procedure imaging was excluded from the analysis.
5-Women with no follow up data

All the exclusion criteria were applied and the cohort was reduced to n=133 for analysis of fibroid/uterine volume change post UAE.
While exclusion criteria (2,3) were not applied when looking at complication rates following UAE as post-UAE imaging was not essential.

4.6.3 Ethical approval:

Caldecott guardian approval was obtained and the local R&D team at the Western Infirmary, Glasgow was approached. Once R&D approved the study, it was uploaded and transferred using the Integrated Research Application System (IRAS) to the next available ethics committee.
The Proportionate Review Sub-Committee of the NRES Committee East of England - Cambridge South reviewed the ethics application on 09 February 2015 and gave a favourable opinion (Appendix 3).

4.7 MRI Images

The standard imaging protocol for pelvic MRI for fibroids in NHS GG&C is T2 weighted Fast Recovery Fast Spin Echo (FRFSE) images acquired in the sagittal and coronal planes (TR 4960ms, TE 85ms, FOV 240mm, 256 x 384 matrix, slice thickness 4mm, echo train length 16). Sagittal T1 weighted Liver Acquisition and Volume Acquisition (LAVA) images were acquired before and after injection of gadolinium contrast agent (Gadovist, Bayer plc, Berkshire, UK). LAVA is a 3D spoiled gradient echo sequence and the following imaging parameters were applied: TR 4.72ms, TE 2.32ms, FOV 280mm, matrix (frequency x phase) 320 x 192, slice thickness 5mm. There was slight deviation from this protocol according to different sites however, all patients in this study had T2 sagittal and coronal planes images. MRI Images were loaded on the picture archiving and communication system (PACS) from the national archive using the Community Health Index number (CHI Number). This is a population register, which is used in Scotland for health care purposes and access is given to medical staff after line manager written approval. The CHI number uniquely identifies a person in this index and is made of 10 characters. The first 6 characters from the left are the patient's date of birth and the remaining four characters are used to differentiate between two individuals with similar dates of birth.

The date of the UAE procedure was checked against the database obtained through CRIS and the dates of the pre-UAE and post UAE MRI was recorded. The time from pre-UAE MRI to event date and from event date to post UAE MRI was calculated. NHS GG&C uses a 6 weeks post procedure outpatient clinic review. This helps identify and address any problems that may arise in the immediate post procedure duration. A further 6 months post procedure MRI is usually organised at this appointment. This is used to evaluate the success of the UAE procedure by measuring shrinkage of the uterus and fibroids, and contrast fill defect in the fibroids. Patients are shown their before and after UAE MRI in a side by side layout for direct comparison and a brief description. This usually re-enforces the success of this procedure in a patient that has symptom improvement as a result of UAE.

4.8 MRI image analysis

Training in MRI image analysis of a fibroid uterus was undertaken. This was supervised by Dr Sue Lassman, Consultant radiologist with special interest in MRI and gynaecology imaging. This was in the form of direct supervised sessions that included measuring fibroid diameters, calculating fibroid volume, assessing the location, calculating uterine diameters, calculating uterine volume and measuring endometrial thickness on MRI images. The basis of contrast enhancement was also learnt. However, this is a subjective observation and would be difficult to reproduce therefore was not used in this thesis. Measurement of the fibroid and the uterus and volume calculations have previously been described (section 2.5).

4.8.1 Calculating shrinkage (change in size after UAE):

The change in the size of the fibroid uterus and the dominant fibroid was assessed and calculated as a percentage rather than absolute numbers due to the wide variation in the size of the uteri and the fibroids measured.

The term “Uterine shrinkage” and “Dominant fibroid shrinkage” were calculated as follows:

Dominant Fibroid shrinkage =
(Vol. of dominant Fibroid post UAE/ Vol. of dominant Fibroid pre UAE – 1) x 100

Fibroid Uterus Shrinkage =
(Volume of fibroid uterus post UAE/Volume of fibroid uterus pre UAE -1) x 100

4.9 Statistics

Descriptive statistics were used when it was appropriate. Paired t-testing and independent T-testing was used to compare parametric data and the results expressed in mean plus/minus standard deviation. One way ANOVA test was used when appropriate with Bonferroni post hoc test when indicated. Spearman's correlation test was used to test association between variables.

Results were statistically significant with p value ≤ 0.05. Details of when each test was used are addressed in each relevant section. Minitab 17 statistical software was used for data analysis and creating graphs that were then edited using Microsoft word 2010 software. Microsoft excel 2010 was also used to draw graphs.

4.10 Results

The number of patients that underwent UAE in 5 years was n=171, after exclusions were made the cohort used in this analysis n= 133 (table 4-1). The timing of the post-UAE MRI was the main exclusion criteria that reduced the cohort number that was analysed. We included women who had the post-UAE MRI at 6 months +/- 6 weeks after the UAE procedure (182 days +/- 42). Therefore, only women who had the post UAE MRI 140 – 224 days after the procedure were included in this section of the analysis.

Table 4-1: Showing the reason why patients were excluded

Reason for exclusion	Number of patients
Post UAE MRI was performed within 140 days (6 months- 6 weeks)	16
Post UAE MI was performed after 334 days (6 months+/- 6 weeks)	11
No post UAE MRI	3
No Pre-UAE MRI	8
Total excluded	**38**

4.10.1 Uterine and fibroid volume

Initially we compared the change in the dominant fibroid volume and uterine volume post-UAE. A paired t-test was used to compare pre-UAE to Post UAE volume of the uterus and the dominant fibroid. This shows that there is a statistically significant reduction in both the mean uterine volume and the mean dominant fibroid volume after a UAE procedure. The mean uterine volume reduced by an average 336.8cm^3 and the mean dominant fibroid volume reduced by an average of 144 cm^3 (table 4-2, fig 4-3 & 4-4).

Table 4-2: The change in uterine volume and dominant fibroid volume

	N	Pre-UAE Mean +/-STD cm^3	Post UAE Mean +/-STD cm^3	P value
Uterine volume	132	882.28 +/- 660.9	545.47 +/- 508.5	< 0.01
Dominant fibroid volume	127	385 +/- 407.2	241.14 +/ -329.6	< 0.01

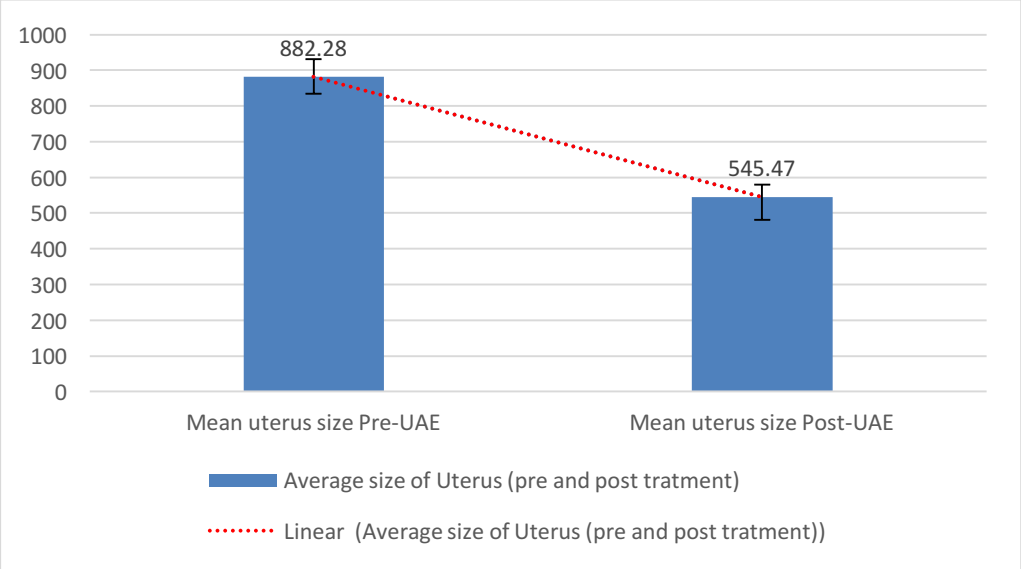


Figure 4-2: The average change in uterine volume

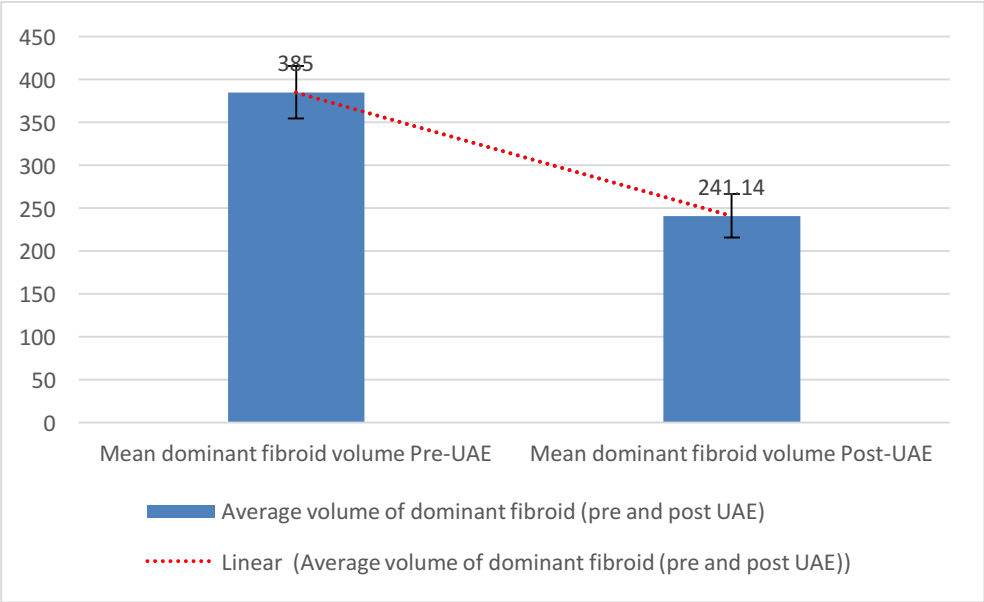


Figure 4-3: The average change in dominant fibroid volume

4.10.2 Large uterus & large dominant fibroids

As mentioned in 4.2.2 a large uterus is that with a volume ≥ 780cm^3 and a large fibroid is that with either a single diameter ≥10cm or a volume of ≥ 700 cm$^{3.}$

An independent sample t–test was used to test for significant differences in treatment effect (shrinkage) between large/small uterus and large/small fibroid.

We found that small fibroids < 700 cm^3 shrunk significantly more than large fibroids ≥ 700 cm^3 ($p < 0.05$). However we could not find a similar effect when comparing large uterine volumes to small uterine volumes. This may be due to a fibroid uterus usually contains multiple fibroids of varying sizes. (Tables 4-3 & 4-4).

Table 4-3: Comparison between small and large fibroids shrinkage

	Shrinkage post-UAE of a small fibroid %	Shrinkage post-UAE of a large fibroid %	P value
Mean shrinkage ±STD	45.74 ± 2.7	34.5 ± 4.72	<0.05

Table 4-4: Comparison between small and large fibroid uterus shrinkage

	Shrinkage post-UAE of a small uterus %	Shrinkage post-UAE of a large uterus%	P value
Mean shrinkage ±STD	37.97 ± 2.67	38.36 ± 3.17	>0.05

4.10.3 Age of the patient

The majority of patients who underwent UAE were between 40 & 50 years with a peak at around 45 years. This is the age that is clinically associated with pre-menopause and period irregularities are common. Around 80% of the women undergoing UAE in this study were above the age of 40. This is the main difficulty that studies which look at fertility face as fertility significantly declines after the age of 35. (fig 4-5, 4-6).

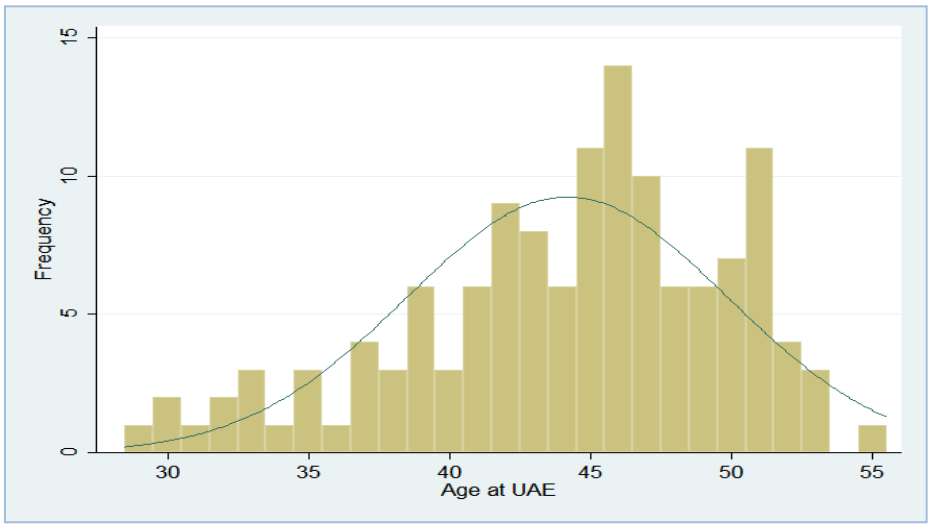


Figure 4-4: Histogram showing the age at time of UAE

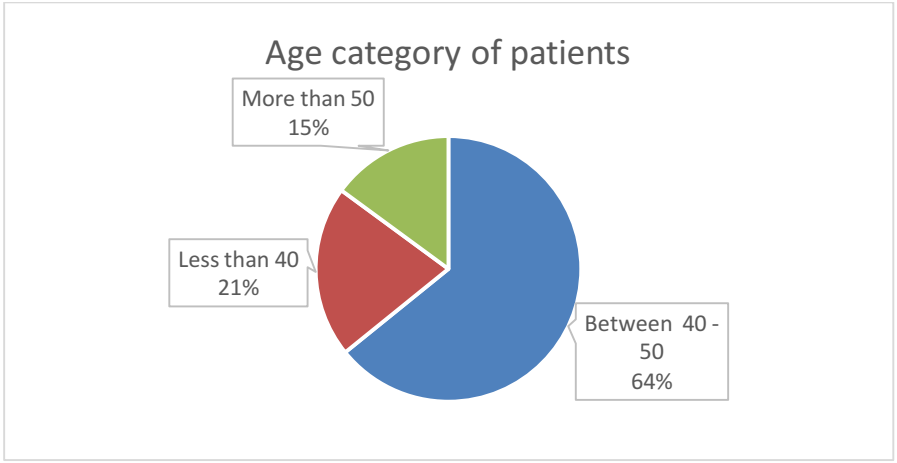


Figure 4-5: Pie chart of age distribution of women undergoing UAE

To assess if there is a relationship between age of the patient and the uterine volume pre-UAE or the dominant fibroid volume pre-UAE we ran a non-parametric correlation test (Spearman's) and the independent variable was age.

Table 4-5: Spearman's correlation of age & pre-UAE volumes

Correlation with age	Number of observations	Rho	P value
Uterus volume pre UAE	133	-0.170	0.053
Dominant fibroid volume pre-UAE	128	-0.162	0.067

There was a weak negative correlation between age and volume of the uterus pre-UAE (rho = -0.170). There was also a weak negative correlation between age and volume of the dominant fibroid pre-UAE (rho = -0.162). This means that with age we expect on average a smaller fibroid uterus and a smaller dominant fibroid. When plotting the age of a woman undergoing UAE vs. the volume of the uterus pre-UAE and the volume of the dominant fibroid pre-UAE, it becomes obvious that the majority of patients are aged between 40-50 years and have a dominant fibroid volume under 500cm^3 and uterine volume under 1000cm^3. (fig 4-7, 4-8)

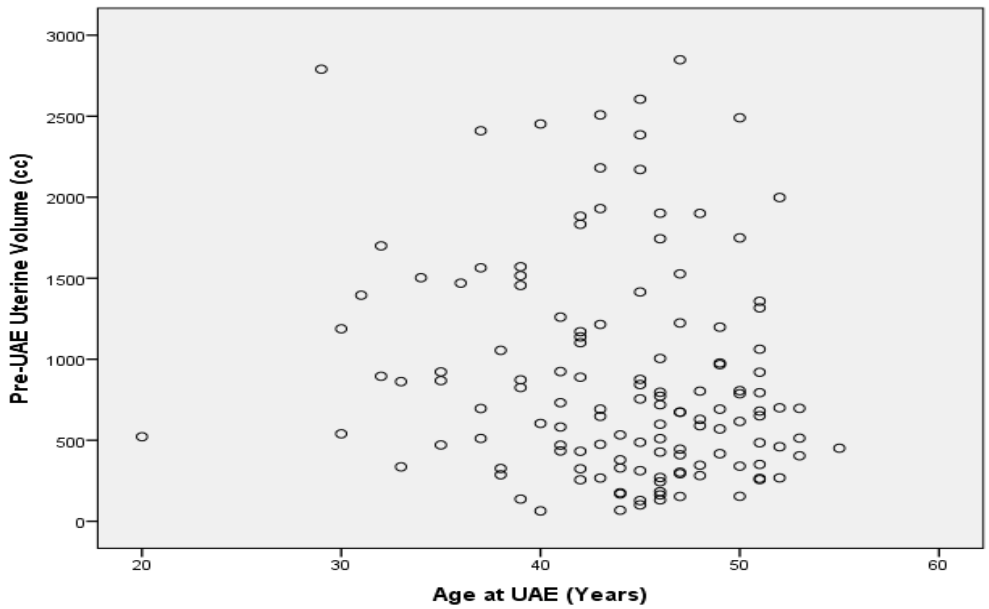


Figure 4-6: Scatter chart of the volume of uterus pre-UAE in relation to age of the patient

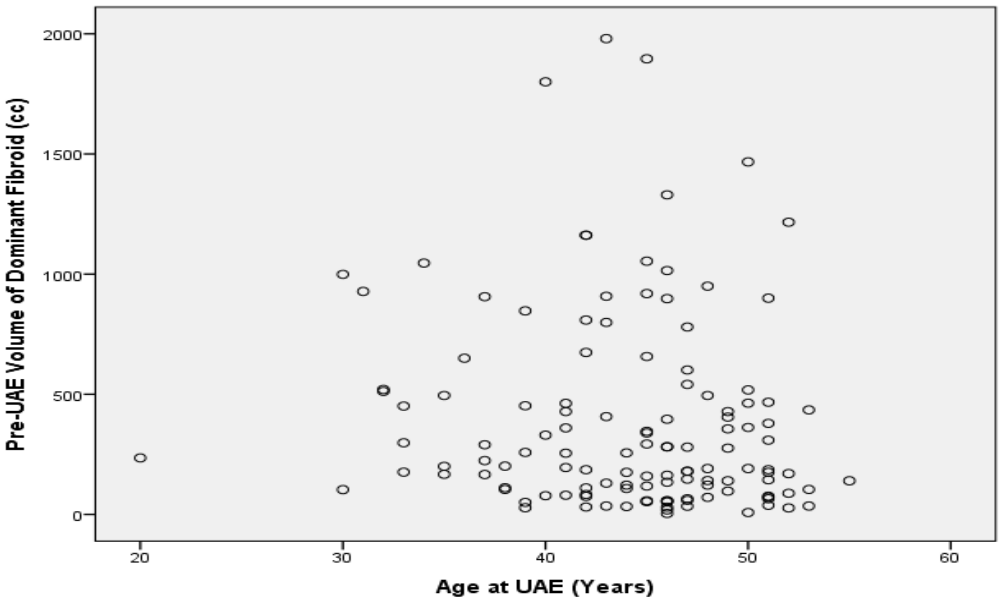


Figure 4-7: Scatter chart showing the volume of the dominant fibroid pre-UAE & age of the patient at the time of UAE

We tested the correlation between age and uterine shrinkage and found this to be weak (rho= 0.127) and statistically insignificant.

When looking at age as a factor affecting shrinkage we found that women in the age group 40-50 years seemed to have the least average shrinkage (response) to UAE. However, those above the age of 50 years had the best response to UAE. This may indicate a synergistic effect of menopause and UAE resulting in maximum shrinkage. (fig 4-9, 4-10).

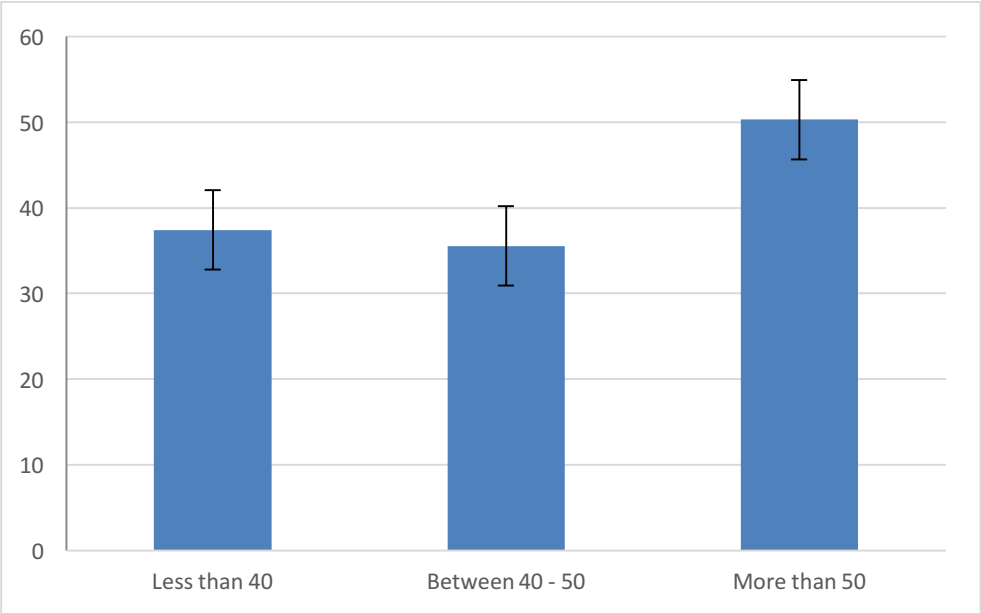


Figure 4-8: Average uterine shrinkage (%) by age group

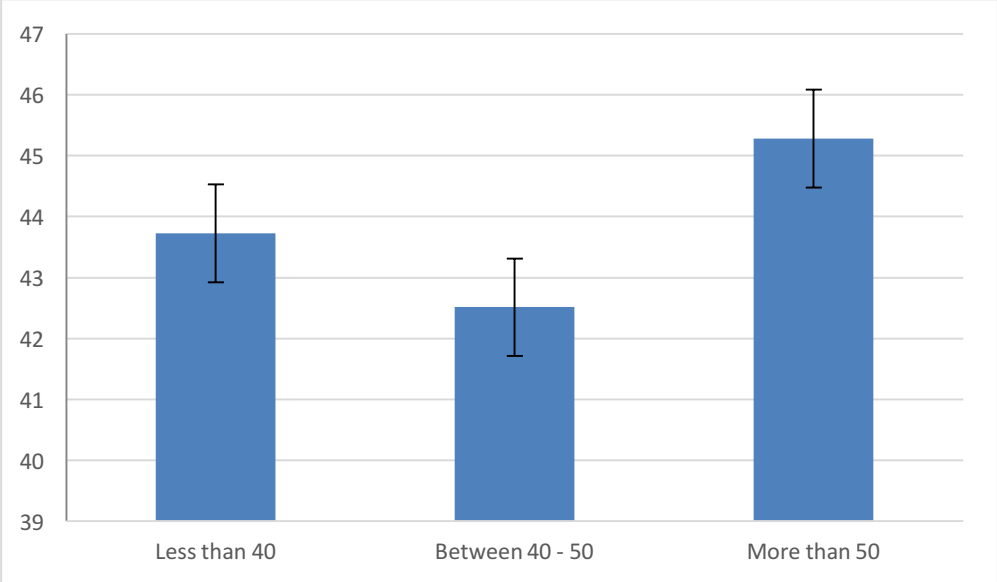


Figure 4-9: Average dominant fibroid shrinkage (%) by age group

4.10.4 Location of the dominant fibroid:

The majority of patients had dominant fibroids that were intramural in location (52.6%). This was followed by subserosal fibroids (31.6%) and then submucosal (6.8%). The total percentage of patients with dominant fibroids that are in contact with the uterine cavity (submucosal or intramural with submucosal element) were 11.3% of the sample.

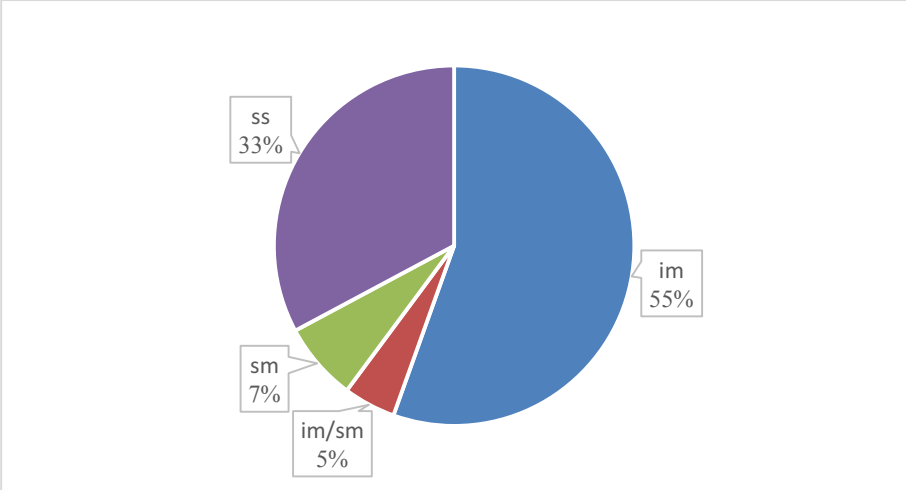


Figure 4-10: Dominant fibroid distribution by location

To test if the dominant fibroid location is a significant factor that plays a role in its shrinkage we used a one-way Anova and post hoc test (Bonferroni) to look at the difference between shrinkage of the dominant fibroid according to different location groups.

Table 4-6: Average dominant fibroid shrinkage according to location

Location of fibroid	N	Shrinkage % Mean ±STD
Submucosal	9	69.63 ± 20.5
Intramural with submucosal element	6	62.29 ± 37.0
Intramural	70	42.98 ± 24.5
Subserosal	42	37.79 ± 27.7

We concluded that shrinkage of the dominant fibroid may be dependent on its location and two group differences reached statistical significance. The first was that submucosal

dominant fibroids shrunk by an average of 26.6% more than intramural dominant fibroids ($p < 0.05$). The second was that submucosal dominant fibroids shrunk by an average of 31.8% more than subserosal fibroids ($p < 0.05$). This is probably due to the way expelled submucosal fibroid volume was calculated as zero & shrinkage as 100% in this study.

Table 4-6 demonstrates that the most shrinkage was seen in dominant fibroids that are submucosal or have a submucosal element followed by intramural fibroids then subserosal fibroids. This suggests an inversely proportionate relationship between proximity of a fibroid to the endometrial cavity and fibroid shrinkage post UAE.

$$\text{Dominant fibroid shrinkage} \propto \frac{1}{\text{Distance from endometrial cavity}}$$

4.10.5 Single & multi-fibroid uterus

Women attend for UAE with a varying number of fibroids in their uterus. Some will have a single fibroid and some will have multiple. It is not yet known if these women will respond in a different manner to UAE. The uteri of women who presented with a single fibroid uterus shrunk on average by 44.1% compared to that of women with a multi-fibroid uterus

which shrunk on average by 35.6%. We compared these two groups using an independent t-test and we found a significant difference ($p < 0.05$). (fig 4-12).

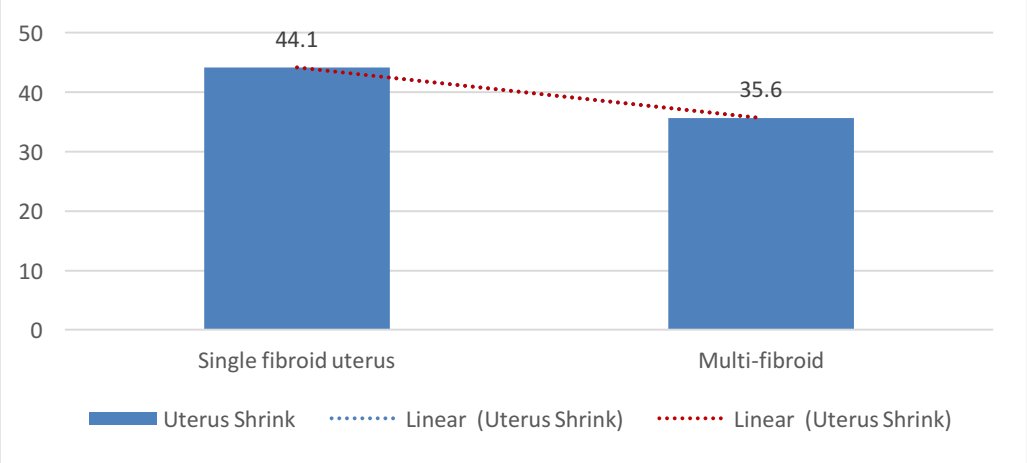


Figure 4-11: Difference in mean uterine shrinkage (%) according to number of fibroid

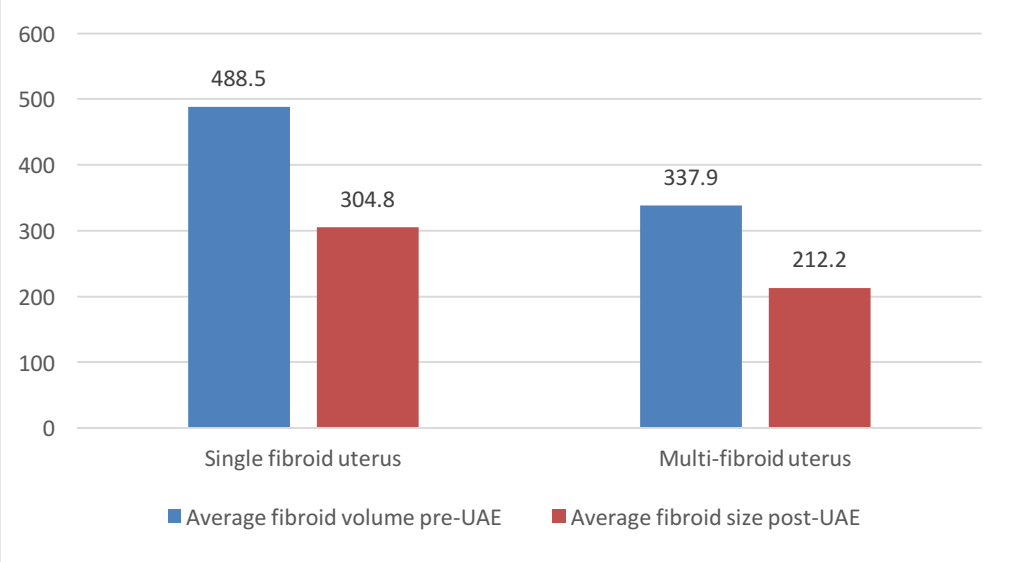


Figure 4-12: The difference in volume shrinkage of dominant fibroids in single fibroid uterus and a multi-fibroid uterus (cm^3)

4.10.6 Complications following UAE

The complication rates after UAE were recorded at 6 months and at 18 months post UAE. However follow up data was not available for 2 patients and a further 2 patients had a repeat UAE procedure throughout the study time line. The second procedure was excluded from the study. This resulted in the total number of patients studied n=167. The timing of the MRI post-UAE was not an exclusion criteria.
The analysis of this section was completed on 09/06/2016 (18 months from when the last patient underwent UAE on 31/12/2014).

4.10.6.1 Six months follow up after UAE

We looked at the re-intervention rates 6 months after UAE and divided them as follows:

a- Hysterectomy
2 patients (1.1%) required a hysterectomy in the first 6 months post UAE. These were both emergency hysterectomies. The first was performed at day 25 post procedure for severe abdominal pain and HMB. The second was performed at day 59 post procedure due to sepsis and the presences of a large necrotic mass in the uterus

b- Myomectomy
1 patient (0.59%) had a re-intervention with a myomectomy within the first 6 months following UAE

c- Repeat UAE
4 patients (2.3%) had a repeat UAE procedure within the first 6 months following UAE

There were also 8 emergency admissions (4.7%) for post embolisation-like syndrome that were all treated conservatively.

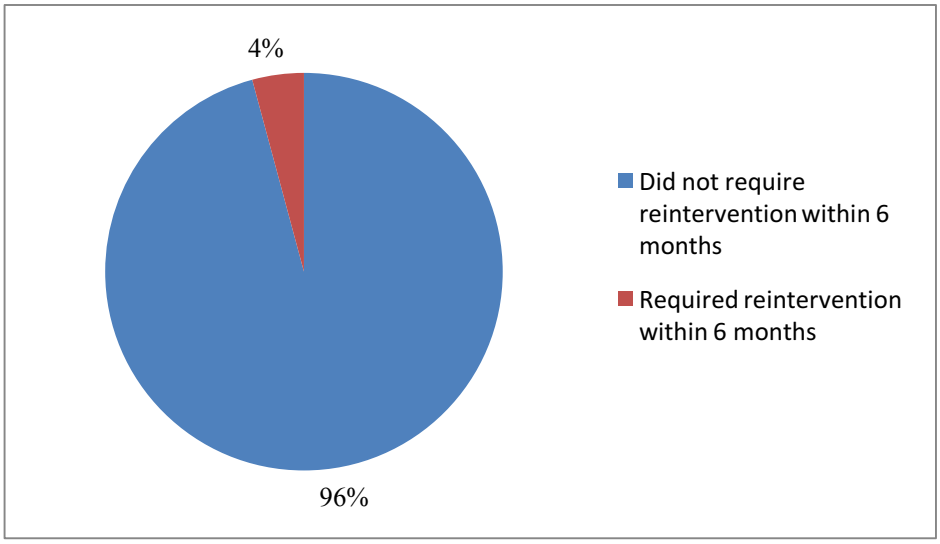


Figure 4-13: Re-intervention rate at 6 months

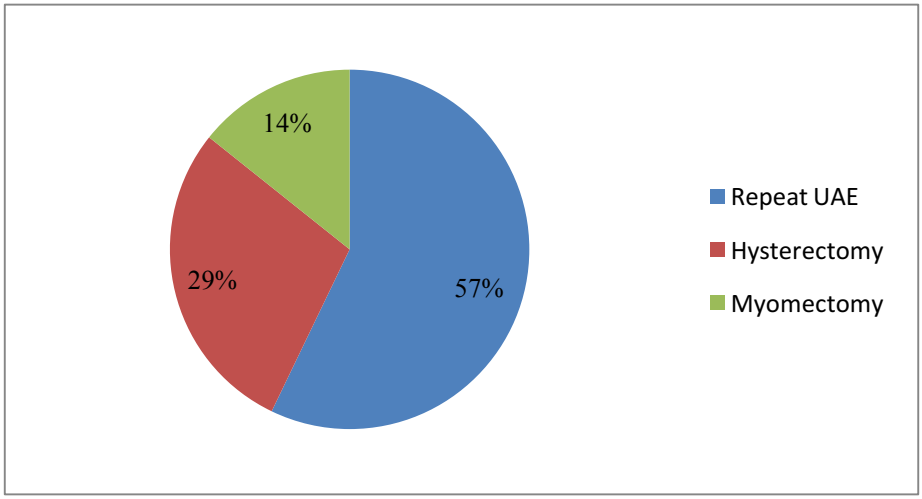


Figure 4-14: Re-intervention by procedures within 6 months (n=7)

4.10.6.2 Eighteen Months follow up after UAE

a- Hysterectomy

11 patients (6.5%) had a re-intervention with a hysterectomy in the first 18 months post UAE

b- Myomectomy

8 patients (4.7%) had a re-intervention with a myomectomy within the first 18 months following UAE

c- Repeat UAE

7 patients (4.1%) had a re-intervention with a repeat UAE procedure within the first 18 months following UAE

Emergency and elective admission within 18 months post UAE:

13 patients (7.7%) required a procedure-related emergency admission within the immediate 18 months post UAE. The main cause of admission was pain.

19 patients (11.3%) required a procedure-related elective admission to the gynaecology department post UAE within the immediate 18 months following UAE. The main reason for this was to undergo re-intervention.

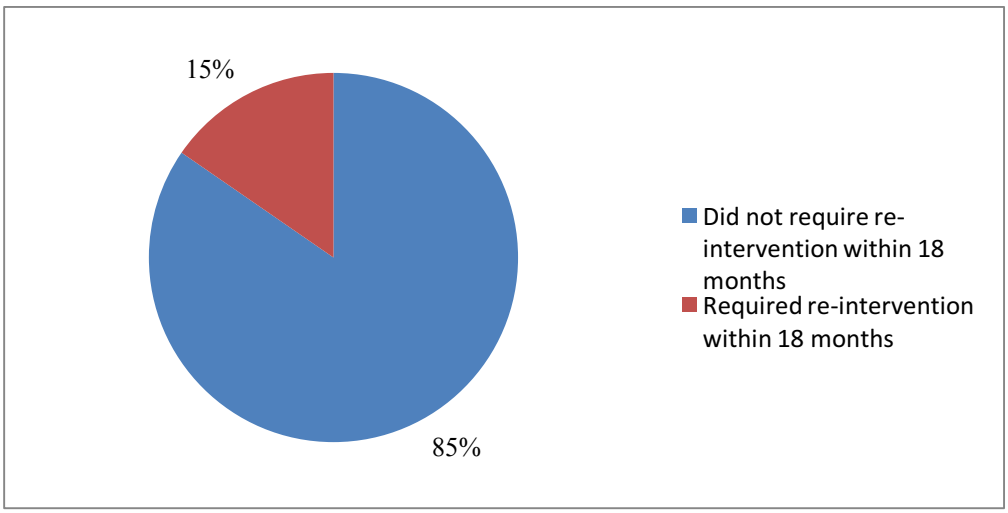


Figure 4-15: Re-intervention rate at 18 months

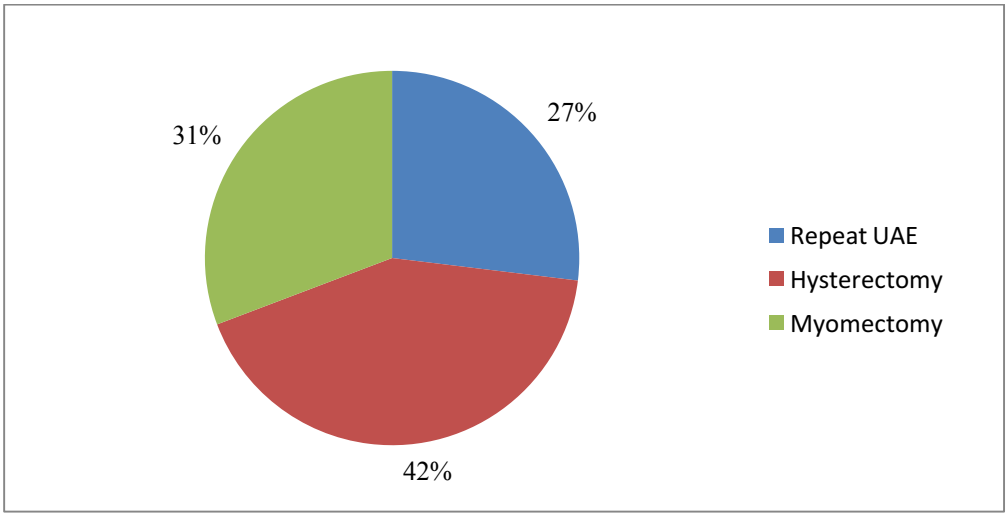


Figure 4-16: Re-intervention by procedures at 18 months (n=26)

4.10.6.3 Comparing 6 to 18 months re-intervention

There was an overall increase in all re-intervention rates at 18 months. The highest rate of intervention was in the form of a hysterectomy. (fig 4-17)

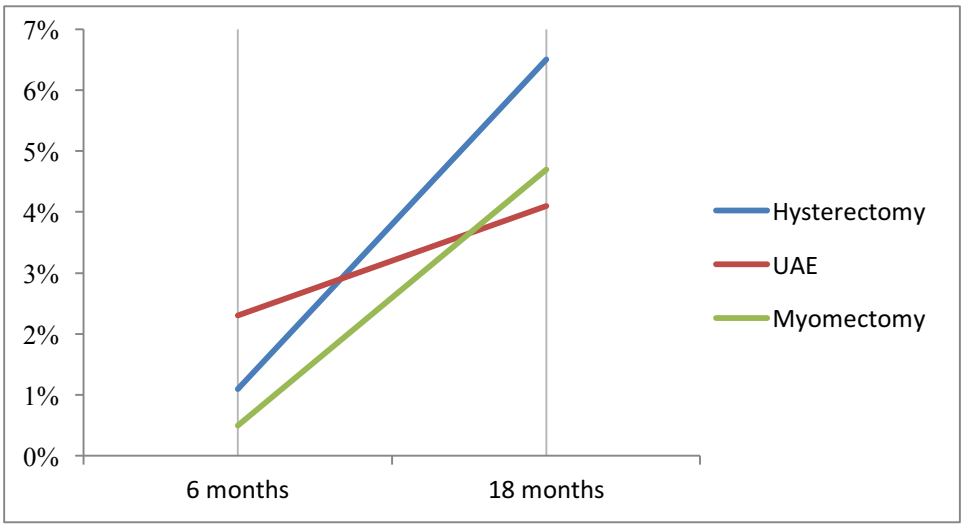


Figure 4-17: Line chart of re-intervention rates

4.10.7 Pregnancy within the 18 months of procedure:

There were a total of 4 pregnancies post-UAE in this study group and conclusions regarding the effect of UAE on future fertility cannot be drawn from this.

Table 4-7: Pregnancy outcomes at 18 months post UAE

Age at UAE	Duration post- UAE until pregnancy	Outcome of pregnancies
30	487 days	Termination of pregnancy (Social reasons)
36	258 days	Miscarriage requiring medical evacuation of retained products of conception
36	53 days	Delivered by elective caesarean section at 38 weeks
31	231 days	Delivered by elective caesarean section at 37 weeks

4.11 Discussion

In most fibroid studies that looked at change in fibroid size, the dominant fibroid measurement was taken into account rather than all fibroids. When this study was started the intention was to measure the volume of all fibroids with any one diameter over 3cm and analyse the changes after UAE. This method was abandoned as it was felt that a fibroid would then be used as an entity devoid of the woman suffering from the symptoms it causes. It also proved time consuming and difficult to gain re-producible data.

After UAE both the uterine volume and dominate fibroid volume significantly reduced in size. Only 5.2% of patients did not have uterine shrinkage and 5.4% did not have dominant fibroid shrinkage. Most interventional radiology units only offer UAE as a treatment for HMB. However, the high success rate of UAE and the percentage of both uterine volume shrinkage and dominant fibroid shrinkage suggest that pressure symptoms caused by a space occupying lesion may also improve. It maybe that a change of practice should be introduced and UAE should also target women that present predominantly with pressure symptoms and offer them an alternative to major surgery.

Patients present to interventional radiology clinics with different uterine and fibroid sizes. It is often perceived by the gynaecologist and even the patient that a large fibroid uterus will not respond to UAE. Although there is no clear consensus of what constitutes a large fibroid uterus or a large fibroid. We used the literature cut off of volume ≥ 780cm^3 to classify a uterus as a large uterus a cut off ≥ 700 cm^3 to classify a fibroid as large. We found that small dominant fibroids < 700ml did indeed shrink significantly more than large fibroids ($p < 0.05$). However we could not find a similar effect when comparing women presenting with large uterine volumes to those with small uterine volumes.

Women around the age of 45y represented the highest percentage of those undergoing UAE. This relatively late presentation when undergoing UAE may be due to some women seeking conservative and non-invasive treatment options prior to UAE. Some woman may feel that UAE is a form of surgery and better postponed until other treatment options have been exhausted. There may be an underlying feeling that if UAE fails then major surgery such as myomectomy or hysterectomy are the only alternative options left to pursue. We found that older women presented on average with a smaller fibroid uterus size and smaller

dominant fibroid. Clinically, this may mean that woman nearer the age of menopause have already started the process of fibroid shrinkage that we expect in post-menopausal women. We found that women aged 50 years and over had the best response to UAE both from uterine volume shrinkage and dominant fibroid shrinkage. This may indicate a synergistic effect of menopause and UAE resulting in maximum shrinkage. We conclude from this that women closer to the age of menopause should not be denied UAE based on age as they seem to be the best responders especially if pressure effect is one of the main symptoms at presentation.

One of the variable aspects of fibroids response to UAE that has not been studied well is the location of the fibroids pre-UAE. The majority of the patients in this cohort presented with an intramural dominant fibroid (55%). We found statistically significant difference in uterine shrinkage between submucosal and intramural fibroids and also between submucosal and subserosal fibroids. This suggests an inversely proportionate relationship between proximity of a fibroid to the endometrial cavity and fibroid shrinkage post UAE. We concluded that fibroids closer to the endometrial cavity (submucosal) tend to shrink more than fibroids further away from the endometrial cavity (subserosal). This is however in keeping with the fact that woman with submucosal fibroids are at higher risk of expulsion of fibroids after UAE which in this study we deemed as 100% shrinkage [276]. Appropriate counselling of such a complication is warranted in women with submucosal fibroids embarking on UAE as a treatment modality.

We also found that patients presenting with a single fibroid uterus responded better than those presenting with a multi-fibroid uterus. This was true for both uterine volume shrinkage and dominant fibroid shrinkage.

The complications and re-intervention rates were looked at for both 6 months and 18 months post UAE. Most studies have not looked at long term outcomes and not followed up patients for more than 6 months. Data was obtained from the Information Services Division for Scotland (ISD) and this was cross-referenced with hospital notes looking at elective and emergency admissions and new clinic referrals post UAE. We found an increase in all re-interventions at 18 months follow-up. When comparing complications rates at 18 months to those at 6 months, hysterectomy increased from 1.1% to 6.5%, myomectomy increased from 0.59% to 4.7% and repeat UAE increased from 2.3% to 4.1%. This amounts to a total re-

intervention rate of 4.1% at 6 months and 15.3% at 18 months. This rate is predicted to rise when further follow up data becomes available [148].

UAE is a safe procedure and the complication rates are low especially serious complications such as a need for emergency hysterectomy [265]. This was found to be 1.1% (2 patients) in our study. The reduction in volume (shrinkage effect) of both the fibroid uterus and the fibroid itself is significant. However, there has been no specific study looking at the effect of UAE on pressure symptoms mainly because UAE was established as a treatment for HMB and any alleviation of pressure symptoms is considered to be an additional benefit rather than a desired consequence.

The safety aspect of UAE with regard to post procedure pregnancy is still a matter of debate and we are eagerly awaiting the results of the FEMME trial that is the first study of UAE that did not exclude women wanting to maintain their fertility. In our study 4 women became pregnant post-UAE and 2 of these women delivered a live baby by elective caesarean section at term.

The main limitation of this study was that it was a retrospective study and that it relied heavily on MRI scans that were performed for pre-UAE assessment rather than within a study context. This resulted in a number of post-UAE MRI scans not being performed within the time limits set by this study and a 6 months +/- 6 weeks approach was necessary to obtain valid comparisons and increase the cohort of the study. Post UAE MRI timing was fed back to the radiology department and improvements were recommended. This limitation led to the exclusion of 38 patients whom had pre or post UAE outside the time limits set by this study. We aim to continue following the patients in our study for a total of 5 years.

5. Chapter 5

Diffusion Weighted Magnetic Resonance Imaging (DWMRI) and its role in UAE

5.1 Diffusion-weighted Magnetic resonance Imaging (DWI)

Diffusion-weighted imaging (DWI) is a non-contrast enhanced functional MRI technique available in the majority of clinical MRI units. It can provide information on the motion of water molecules in tissues. It permits the quantitative evaluation of tumour tissue such as fibroids. This is through a quantitative parameter, called apparent diffusion coefficient (ADC). The ADC maps that are generated using DWI provide accurate quantification of the cellular motion of water molecules[292]. This movement is dependent on the cellularity of the tissue/lesion allowing tissue characterisation, and possibly reflecting cell death [293]. A simplistic, though conveniently useful concept is that ADC values are inversely related to tumour cellularity. That is, tissues having relatively high cell density tend to exhibit lower ADC values due to the impeded water movement amongst the cell-packed environment. Treatment which leads to cell necrosis such as UAE will therefore alter cell membrane permeability and water homeostasis, leading to changes in tumour cell density and changes to ADC [294]. The changes in DWI signal and ADC results can only be hypothesized, as there is no gold-standard procedure which measures water movement between cells. The underlying physical phenomena which contribute to loss of signal on diffusion- weighted images remain incompletely understood.

Benign pathological processes such as uterine fibroids may alter cellular structure and membranous permeability, with ultimate shifting of water molecules between tissue compartments [295]. ADC measurements of tissues or lesions may serve as a clinical follow-up tool after medical or locoregional therapy such as UAE [296].

A small number of studies have attempted to predict the volumetric response of fibroids following UAE using DWI MRI. *Liapi et al* [297] looked a 32 fibroids in 11 patients and concluded that ADC maps obtained from DWI MRI may serve as an imaging tool for assessing treatment response after UAE. *Hecth et al* [298] replicated this by retrospectively looking at 28 leiomyomas in 11 women who underwent UAE. These women had a pre-UAE MRI and a follow up MRI > 120 days post procedure. They found Pre-UAE ADC values correlated significantly with percent volume response following UAE. On the other hand a further study of 17 women (27 fibroids) found that DWI MRI and ADC reflected early and delayed changes in fibroids after UAE but they were not able to significantly demonstrate a relationship with the outcome [299].

5.2 Diffusion weighted MRI technique and image analysis:

Stejskal and Tanner first described spin echoes in the presence of a time dependent magnetic field gradient in 1965 [300]. This was later the basis of developing DWMRI. It involves a modified spin-echo T2 MRI sequence, to which a symmetrical pair of diffusion sensitising gradients is applied around the 180 degree refocusing pulse. Static molecules obtain phase information from the first diffusion gradient, but this information is then re-phased by the second gradient without a change in the measured signal intensity. Mobile water molecules acquire different phase information from the first gradient, but, because of their motion, their signal will not be completely re-phased by the second gradient, leading to a signal loss. Hence, the motion of water molecules is detected as attenuation of the measured signal intensity on DWI. The sensitivity of the DWI sequence to the movement of water molecules can vary by changing the gradient amplitude, the duration of the applied gradient and the time interval between the paired gradients. Collectively this can change the diffusion sensitivity and is known as the b-value. This is directly proportional to the three factors listed above [243]. To quantitatively characterise the DWI images, they are acquired with at least two different b-values. Usually b= zero and a larger b value (250, 500, 1000). The higher the b-value the more accurate the ADC value is to detect water motion at the expense of image clarity leading to image distortion (fig 5-2). The slope of a logarithmic plot between the b-values on the *x-axis* and the relative signal intensity on the *y-axis* gives us the apparent diffusion coefficient (ADC) value in units of s/mm^2 (fig 5-1).

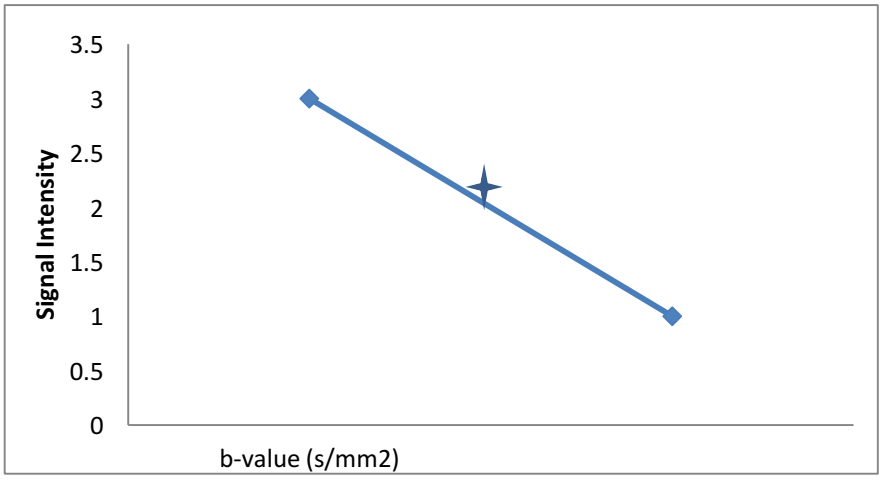


Figure 5-1: ADC Value calculation (slope of log between b-value and signal intensity

DWI is a well-established technique in cerebral imaging [301]. However, its application to imaging of other parts of the body is new. It is now being used as a part of the imaging protocols for pelvic malignancies and fibroids [302]. The aim of this study was to assess whether using DWI offered any additional information over a standard MRI pelvis when imaging uterine fibroids. We wanted to assess if the ADC value can be used as a predictor of the response of the fibroid uterus to UAE and if it could improve the safety profile of UAE.

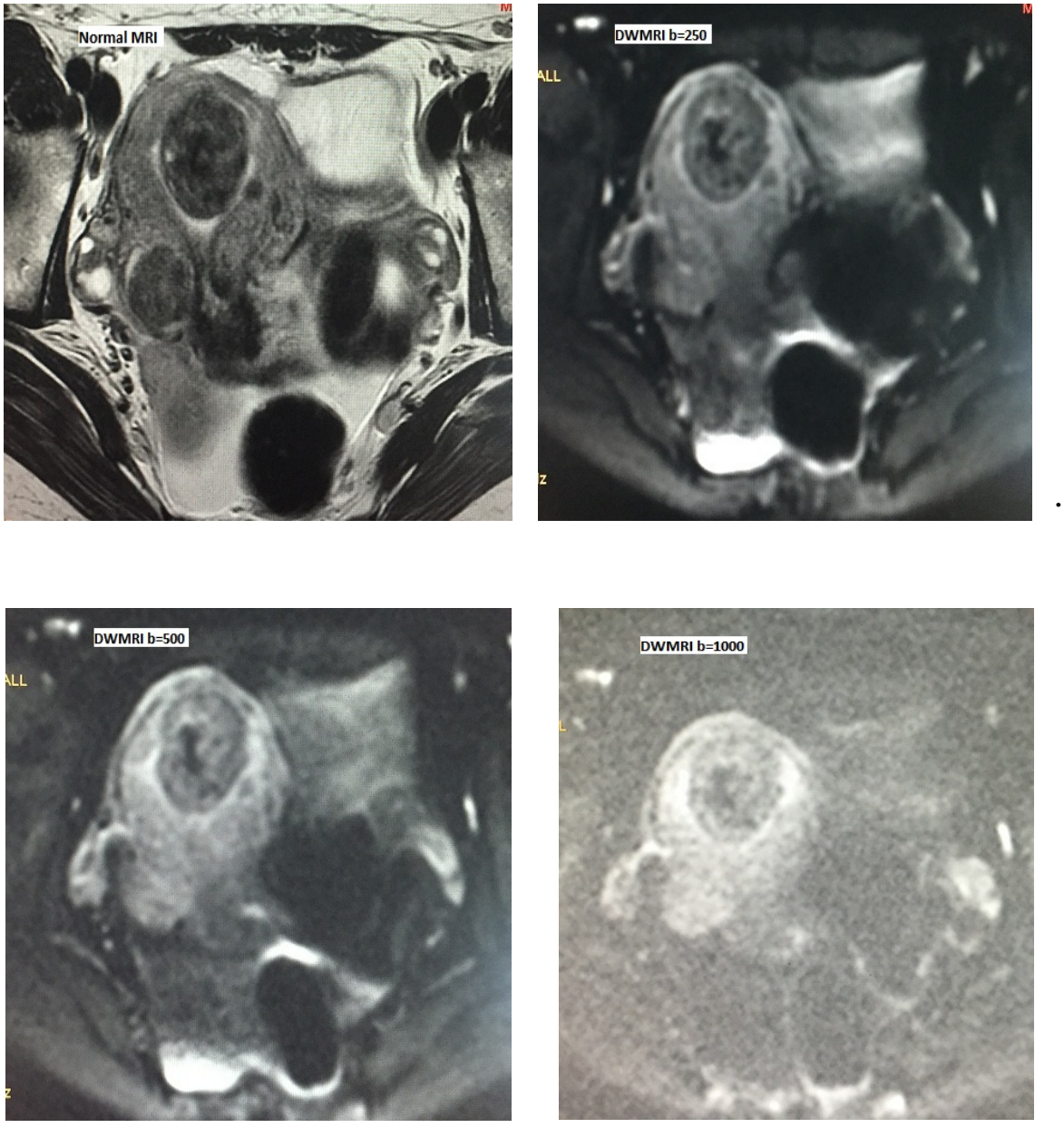


Figure 5-2 Differences between a normal MRI (upper left) and a DWMRI at b=250 (upper right), b=500 (lower left) and b=1000 (lower right)

5.3 Uterine artery Embolisation procedure:

UAE was carried out by one of three experienced interventional radiologists using a standard technique. This involved a right common femoral artery puncture and each uterine artery selected with a 2.7 French co-axial catheter (Progreat, Terumo UK Ltd, Surrey, UK.) and embolisation carried out on each side to complete stasis. The embolisation agent used was either Spongostan or embosphere.

Spongostan Absorbable Haemostatic Gelatin Sponge (Ethicon Medical Ltd. Livingston, Scotland) was manually cut into approximately 1mm squares and mixed with iodinated contrast (Omnipaque 300) to form a 'slurry' using a 5-10cc reservoir syringe and a 1-2cc delivery syringe connected via a three way tap. The residual sponge was weighed to quantify the amount used. Embospheres (Merit Medical Ltd, Coatbridge, Scotland) were used according to the manufacturer instruction for use namely mixed with an equal volume of contrast and the first two ampoules on each size being size 500-700 microns and if more was needed then the particle size was increased to 700-900 microns. Pain was managed using an agreed hospital protocol (appendix 4).

The standard imaging protocol was as follows: T2 weighted Fast Recovery Fast Spin Echo (FRFSE) images acquired in the sagittal and coronal planes (TR 4960ms, TE 85ms, FOV 240mm, 256 x 384 matrix, slice thickness 4mm, echo train length 16). Sagittal T1 weighted Liver Acquisition and Volume Acquisition (LAVA) images were acquired before and after injection of gadolinium contrast agent (Gadovist, Bayer plc, Berkshire, UK). LAVA is a 3D spoiled gradient echo sequence and the following imaging parameters were applied: TR 4.72ms, TE 2.32ms, FOV 280mm, matrix (frequency x phase) 320 x 192, slice thickness 5mm.

5.4 Methods

A pilot study was conducted in one large tertiary referral hospital in the UK. The aim was to compare two embolic agents (Gelfoam and Embosphere) used in UAE. 20 Patients were randomised between March 2011 and January 2012 to receive either embolic agent and 6 month follow up was complete in August 2012.

The study was approved by the regional ethics committee. All patients were provided with a written information sheet at least 24 hours in advance of being consented. Patients were initially seen at an Interventional radiology clinic and a 1 month and a 6 months follow up took place at the same clinic. Each patient underwent a Pre-UAE, 24 hour post UAE and a 6 months Post UAE DWMRI. The study was funded by a research grant from the British Society of Interventional Radiology. Data in this chapter relates only to the DWI imaging of all 20 patients in this study. Although direct comparison between the two groups was made in relation to uterine volume and dominant fibroid shrinkage, this was not the case for DWI analysis. It was felt that comparing baseline DWI MRI to post procedure MRI of the whole group would help answer the questions set out earlier in this chapter.

5.5 Study Population:

Women at least 18 years old were eligible if they had one or more fibroids of more than 2cm diameter causing HMB as the main symtpom. Exclusion criteria included a contraindication to contrast enhanced magnetic resonance imaging (CEMRI), recent or ongoing pelvic infection, severe allergy to iodinated contrast media and the presence of significant other pelvic pathology e.g. adenomyosis.

5.6 Image analysis

All analysis was carried out on a GE workstation (ADW 4.0; GE Healthcare). Only the dominant fibroid was evaluated in each patient. The following variables were assessed pre-UAE, 24 hours post UAE and 6 months post-UAE: uterine volume, dominant fibroid volume, endometrial thickness, ADC measurement of dominant fibroid, myometrium and when possible for the endometrium.

5.6.1 **Analysis of diffusion-weighted images**

A GE MRI workstation at the Beatson West of Scotland Cancer Centre in Glasgow was used. Images for each patient were loaded from the MRI central server onto the GE workstation. The b=0, b=250, b=500 and b=1000 diffusion images were analysed using FuncTool v. 4.4.05 software (GE Healthcare) on the GE workstation and used to generate a corresponding ADC map (fig 5-4 & 5-5). To calculate the ADC value, a circular region of interest (ROI) was drawn encompassing the dominant fibroid, but taking care to avoid the myometrium (to avoid any partial volume averaging), and an ADC value was calculated. From the same data set, ADC values were also calculated for the myometrium and when possible for the endometrium. The ROI for this was used at the same location of the uterus on the pre- and post-embolisation scans. The ROI for the fibroid and myometrium varied between 10mm^2 and 30mm^2, depending on fibroid size and the myometrial thickness. The ROI for the endometrium varied between 2mm^2 and 4mm^2, depending on the endometrial thickness (fig 5-3).

5.6.1 **Volume measurements**

The dimensions of the fibroids were measured along the anteroposterior (AP), craniocaudal (CC) and the transverse long axis (TR). The volume of the dominant fibroid was calculated using a standard prolate ellipse formula (AP x CC x TR x 0.5233) [177]. This same method was used to calculate the volume of the uterus incorporating the fibroid/fibroids (section 2.5).

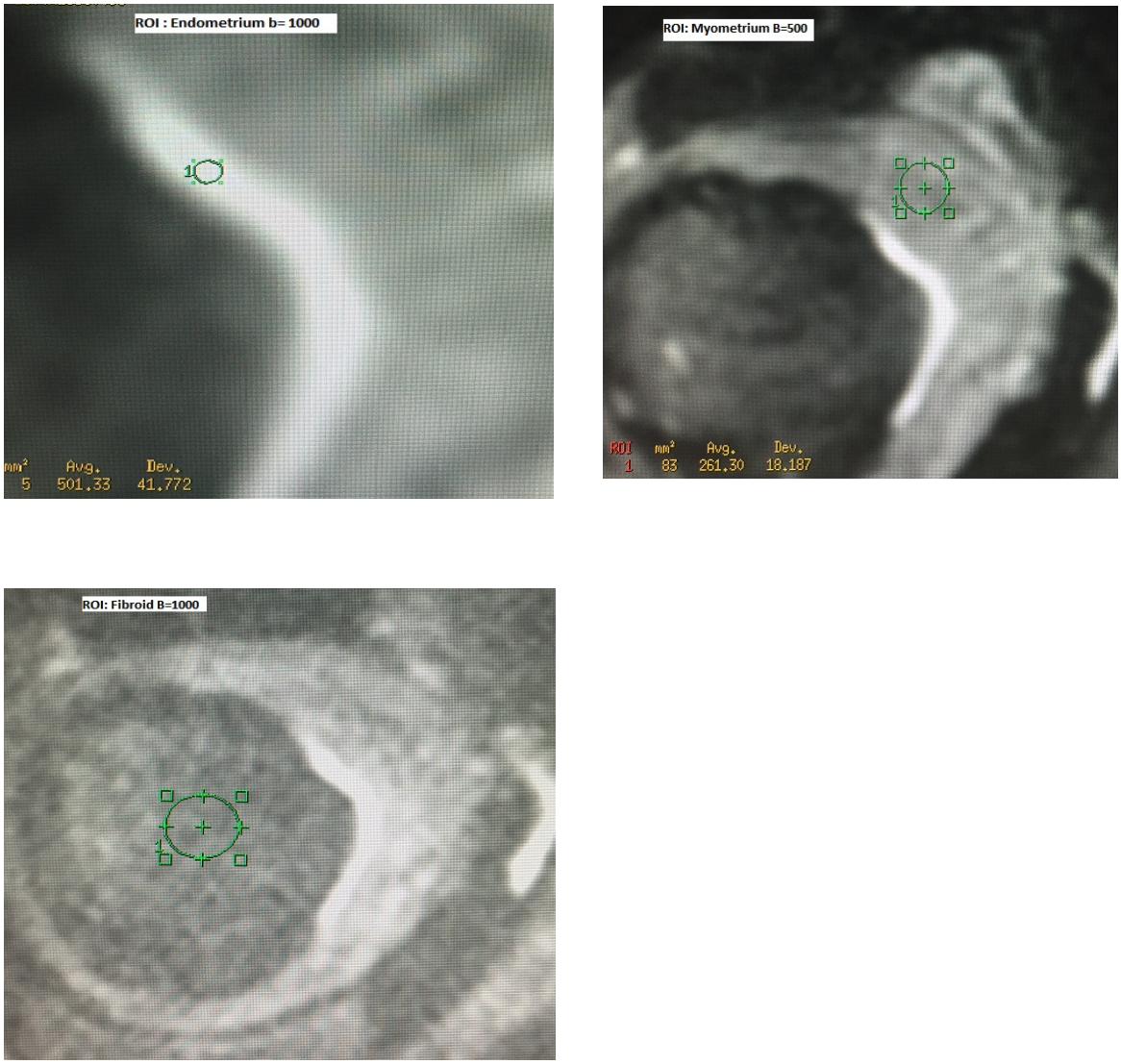


Figure 5-3 Calculation of ADC value by selecting a region of interest (ROI). Fibroid (upper left), Myometrium (upper right) and endometrium (lower left)

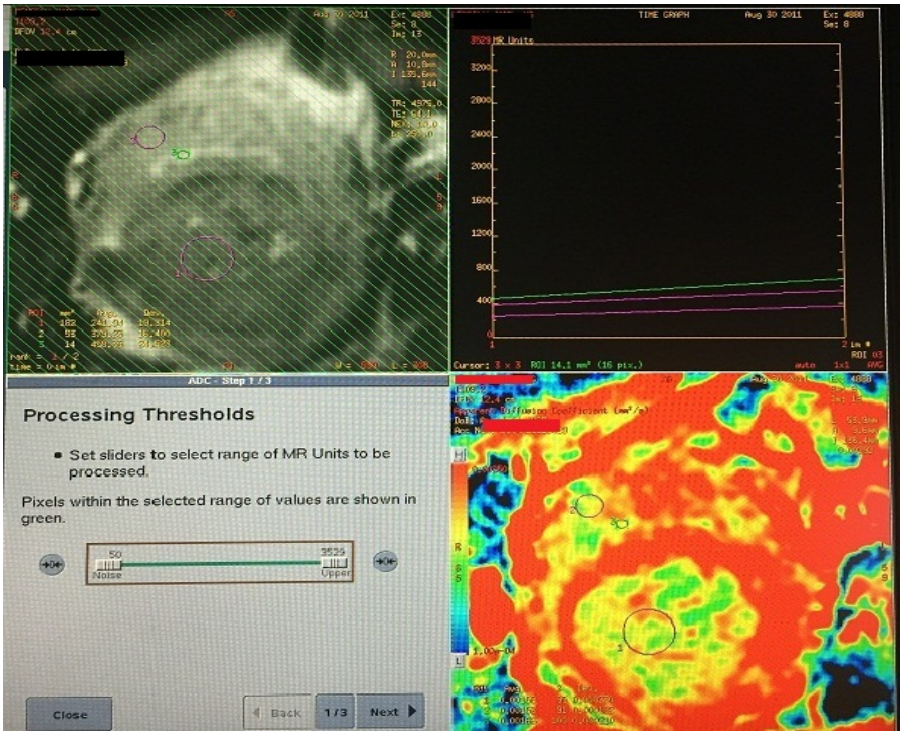


Figure 5-4 Functool software window showing DWI view, ADC curve & ADC colour map

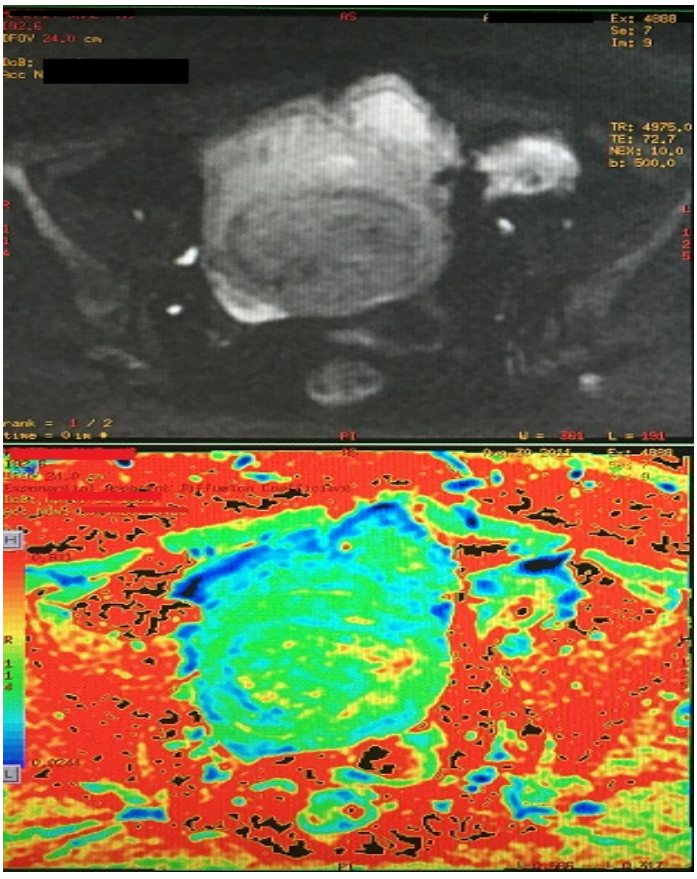


Figure 5-5 DWMRI and ADC colour map to assist in finding ROI

5.7 Statistics

Paired t-test and Mann Whitney test were used to assess changes in paired samples. Pearson's correlation was used to assess the relationship between groups of data. Minitab 17 statistical software package was used to for analysis and drawing graphs. Some graphs were drawn using Microsoft excel.

5.8 Results

As both the change in uterine volume and dominant fibroid volume post-UAE were significant ($p < 0.05$), the results of ADC analysis were grouped as pre & post-UAE ADC values rather than basing them on the embolic agent used (n=20).

Table 5-1: summary of statistics

The Character	N= 20
Age	Range 32-53 44.5 ± 5.64
Pre-UAE Uterine Volume	932mm^3 ± 669mm^3
6 months Post UAE Uterine Volume	656mm^3 ± 547 mm^3
Uterine volume shrinkage (all)	N=20 219.6 mm^3 ± 305.8 mm^3
Uterine Volume shrinkage (only those that shrunk)	N= 16 315.9 mm^3 ± 253.9 mm^3
Location of largest fibroid Submucosal Subserosal Intramural	 n= 2 n= 5 n= 13
Number of fibroids for each patient Single Two (multiple) >2	 N= 7 N= 2 N= 11
Pre-UAE volume of dominant fibroid	441 mm^3 ± 494 mm^3
6 months post UAE volume of dominant	314 mm^3 ± 406 mm^3

fibroid	
Dominant fibroid shrinkage (all)	216.9 mm^3 ± 245.7 mm^3
Dominant fibroid shrinkage (only fibroids that shrunk)	232.1 mm^3 ± 242.5 mm^3
Single fibroid uterus shrinkage	231 mm^3 ± 168.2 mm^3
Multiple fibroid uterus shrinkage	93.6 mm^3 ± 122.5 mm^3

5.9 Uterine volume and dominant fibroid volume:

This randomized trial comparing Gelfoam with Embospheres was intentionally designed as a pilot study to compare the outcome of using these two embolic agents. There was insufficient data in the literature on which to base a power calculation for a definitive study. The only significant difference found between the two groups was in the reduction of uterine volume at 6 months post UAE which amounted to 281cm^2 in favour of Embospheres ($p<0.01$). When combining both groups together there was a significant reduction in both uterine volume and dominant fibroid 6 months post-UAE compared to baseline (table 5-2).

Table 5-2: Change in uterine volume and dominant fibroid volume

	Mean Pre-UAE cm^3	Mean Post-UAE cm^3	Average shrinkage	P
Uterus volume	907.84	688.27	24.2%	< 0.05
Dominant fibroid volume	462.47	330.04	29.1%	< 0.05

5.10 The dominant fibroid

5.10.1.1 Change in ADC value

We found a significant reduction in ADC values of the dominant fibroids between baseline (pre-UAE) and 6 months post-UAE for each level of (B) sensitivity. This indicates that this method can detect the changes that occur in the dominant fibroid. These measurements are objective and reproducible.

Table 5-3: Pre & Post-UAE ADC values at different levels of sensitivity

Sensitivity b=	Pre-UAE Mean ± stand dev	6m Post-UAE Mean ± stand dev	p-value
250	0.001674 ± 0.000356	0.001425 ± 0.000504	< 0.01
500	0.001372 ± 0.000393	0.001106 ± 0.000469	< 0.01
1000	0.000940 ± 0.000335	0.000716 ± 0.000450	< 0.05

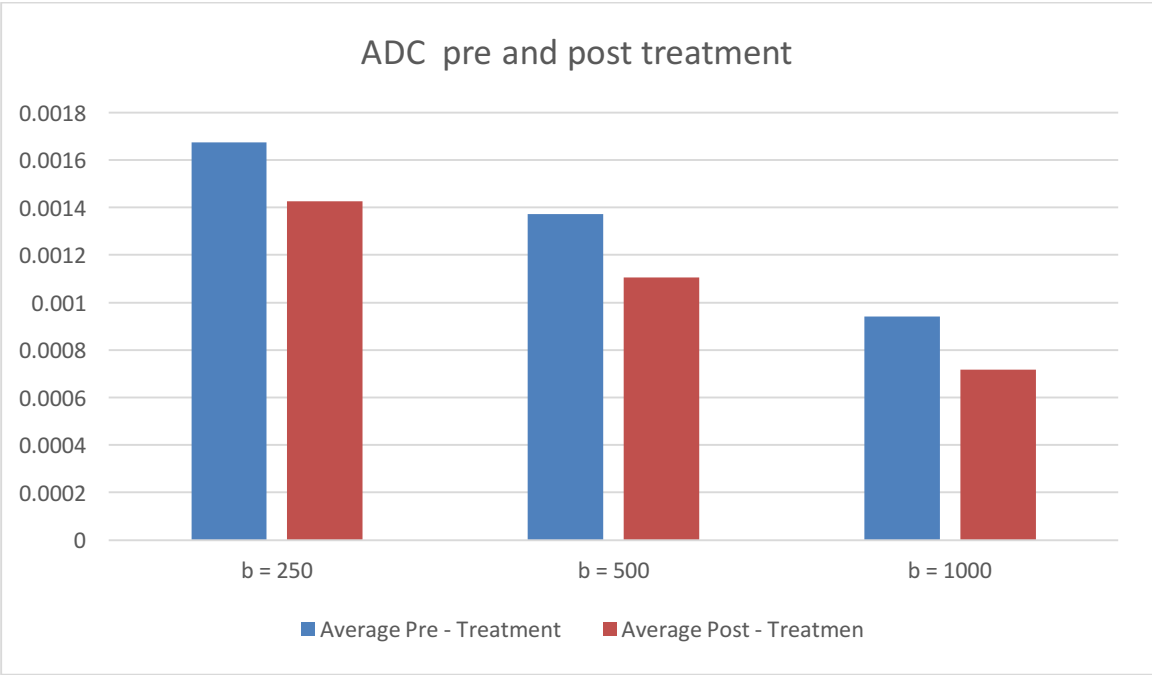


Figure 5-6: The change in Pre-UAE & Post-UAE ADC values at different levels of sensitivity

5.10.1.2 ADC value pre & post UAE

We tested the correlation between pre-UAE ADC values and Post-UAE ADC values of the dominant fibroid. We found a moderately positive and statistically significant correlation at all levels of sensitivity (Table 5-4 & fig 5-7). This indicates that knowing the ADC value of the dominant fibroid pre-UAE may predict the ADC value of that fibroid post-UAE. Clinically this may be used as a predictor of the response to UAE. It could also alleviate the need for contrast material in those patients with a sensitivity/contraindication to contrast. As the change in ADC values can be used as an objective measurement of fibroid necrosis (death) rather than the subjective change in contrast uptake that is widely used.

Table 5-4: Pearson's correlation between pre-UAE & post UAE ADC values of the dominant fibroid (* ρ is Pearson's correlation coefficient value)

Level of sensitivity (b=)	Pearson's Correlation between pre-UAE ADC and post-UAE ADC & p value	P value
250	*ρ = 0.55	0.01
500	ρ = 0.71	< 0.001
100	ρ = 0.68	0.001

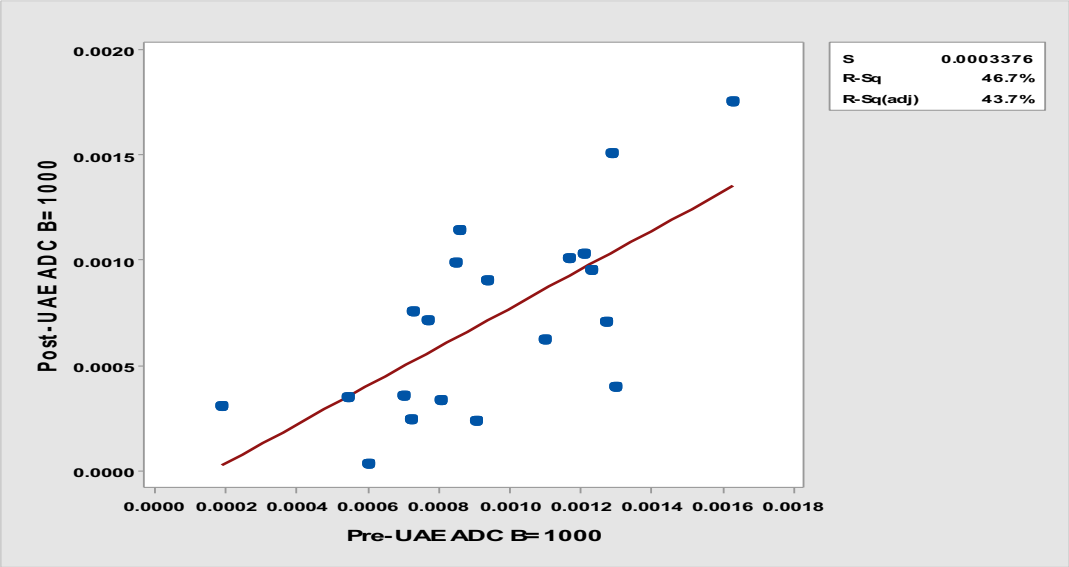


Figure 5-7: Linear regression showing the correlation between pre & post UAE ADC values of the dominant fibroid

5.10.1.3 Dominant fibroid volume reduction

Using Pearson's correlation, we found a statistically significant correlation between dominant fibroid volume reduction and both pre-UAE and post-UAE ADC values, for all levels of sensitivity. However, the relationship was much stronger between dominant fibroid volume reduction and pre-UAE ADC values. This points further to the possibility of predicting how much the dominant fibroid would shrink based on its ADC value.

Table 5-5: Correlation of pre/post UAE ADC and dominant fibroid volume reduction at different levels of sensitivity

ADC Sensitivity (b=)	Correaltion of Dominant fibroid volume reduction with Pre-UAE ADC	Correaltion of Dominant fibroid volume reduction with Post-UAE ADC
250	$\rho = 0.736$, $p < 0.001$	$\rho = 0.44$, $p = 0.05$
500	$\rho = 0.713$, $p < 0.001$	$\rho = 0.51$, $p = 0.02$
1000	$\rho = 0.722$, $p < 0.001$	$\rho = 0.4$, $p = 0.03$

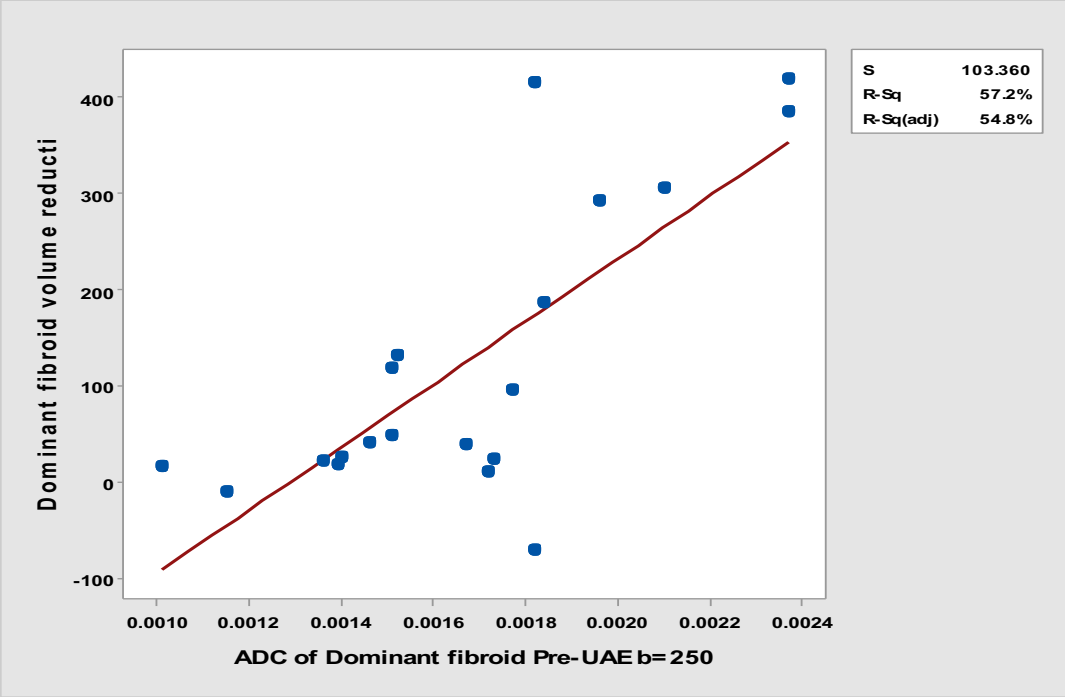


Figure 5-8: Linear regression showing the relationship between pre-UAE ADC values of the dominant fibroid at b=250 and the dominant fibroid volume reduction

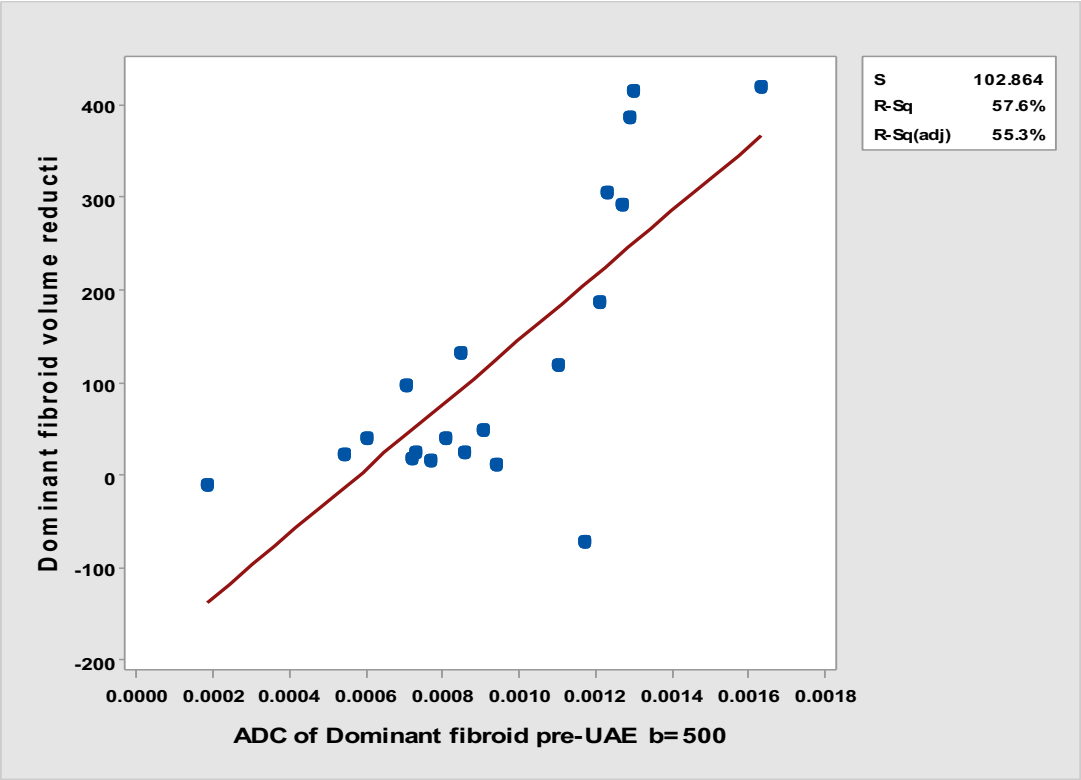


Figure 5-9: Linear regression showing the relationship between pre-UAE ADC values of the dominant fibroid at b=500 and the dominant fibroid volume reduction

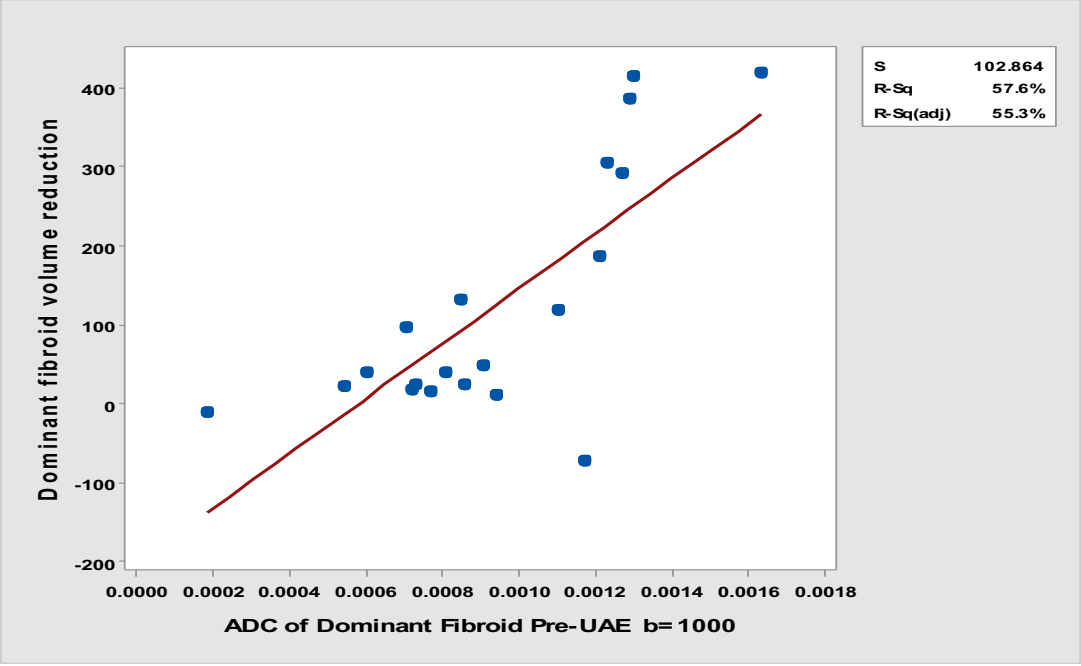


Figure 5-10: Linear regression showing the relationship between pre-UAE ADC values of the dominant fibroid at b=1000 and the dominant fibroid volume reduction

5.10.1.4 Dominant fibroid shrinkage

Using Pearson's correlation, we found a weak correlation between dominant fibroid shrinkage and pre/post UAE ADC values, for all levels of sensitivity. However, these did not reach statistical significance.

Table 5-6: Correlation of pre/post UAE ADC value and dominant fibroid shrinkage at different levels of sensitivity

ADC Sensitivity b=	Correaltion of Dominant fibroid shrinkage with Pre-UAE ADC	Correaltion of Dominant fibroid shrinkage with Post-UAE ADC
250	$\rho = 0.17$ $p = 0.46$	$\rho = 0.22$ $p = 0.33$
500	$\rho = 0.29$ $p = 0.2$	$\rho = 0.29$ $p = 0.21$
1000	$\rho = 0.18$ $p = 0.44$	$\rho = 0.22$ $p = 0.34$

We conclude from this that both Pre-UAE ADC values and post-UAE ADC values of the dominant fibroid positively correlate with dominant fibroid volume reduction at all sensitivities. These are absolute values of volume derived by deducting the post-UAE fibroid volume from the pre-UAE fibroid volume.

When this volume reduction was changed into a percentage using shrinkage as a response, we could not find any correlation with either the pre or post UAE ADC values for all levels of sensitivity.

5.11 The fibroid uterus

Similar analysis was performed looking at the relationship between the ADC of the dominant fibroid and the volume of the uterus and its response to UAE.

5.11.1 Uterine volume reduction

It is not possible to calculate the ADC value of the fibroid uterus as it is composed of different layers and sometimes multiple fibroids. We therefore looked at the correlation between the ADC value of the dominant fibroid and the change in the uterine size. We found no statistically significant relationship between the uterine volume reduction and the pre/post UAE ADC value of the dominant fibroid at all levels of sensitivity (table 5-7 & fig 5-11).

Table 5-7: Correlation between fibroid uterus volume reduction and pre/post UAE ADC of the dominant fibroid at different levels of sensitivity

ADC Sensitivity b=	Correlation between uterine volume reduction & Pre-UAE ADC of dominant fibroid	Correlation between uterine volume reduction & Post-UAE ADC of dominant fibroid
250	ρ = 0.19 p= 0.4	ρ =0.023 p= 0.9
500	ρ = 0.38 p= 0.09	ρ =0.076 p= 0.74
1000	ρ = 0.45 p= 0.78	ρ =0.065 p= 0.78

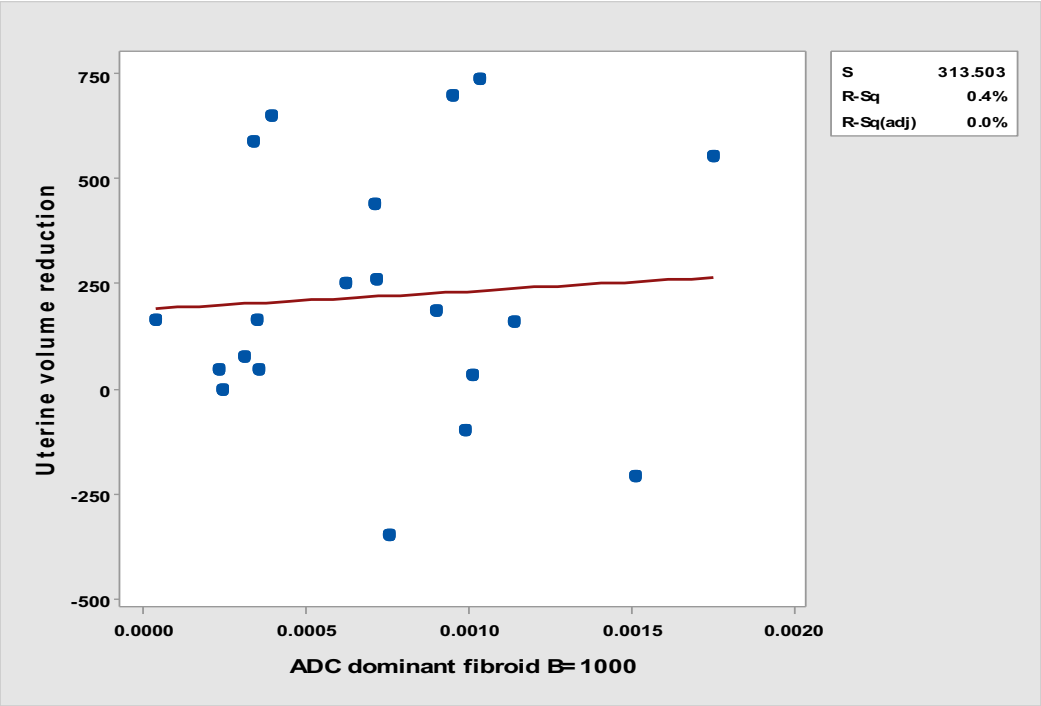


Figure 5-11 Line plot showing no correlation between uterine volume reduction and Pre-UAE ADC of the dominant fibroid at b=1000

5.11.1 Uterine shrinkage

We looked at the correlation between uterine shrinkage and the Pre-UAE dominant fibroid ADC value and found no statistically significant relationship at all levels of sensitivity (table 5-8 & fig 5-12).

Table 5-8: Correlation between uterine shrinkage and pre/post UAE ADC of the dominant fibroid at different levels of sensitivity

ADC Sensitivity b=	Correlation between uterine shrinkage and Pre-UAE ADC of dominant fibroid	Correlation between uterine shrinkage and Post-UAE ADC of dominant fibroid
250	ρ = -0.21 p = 0.36	ρ = -0.33 p = 0.15
500	ρ = -0.1 p = 0.67	ρ = -0.22 p = 0.34
1000	ρ = 0.01 p = 0.96	ρ = -0.2 p= 0.39

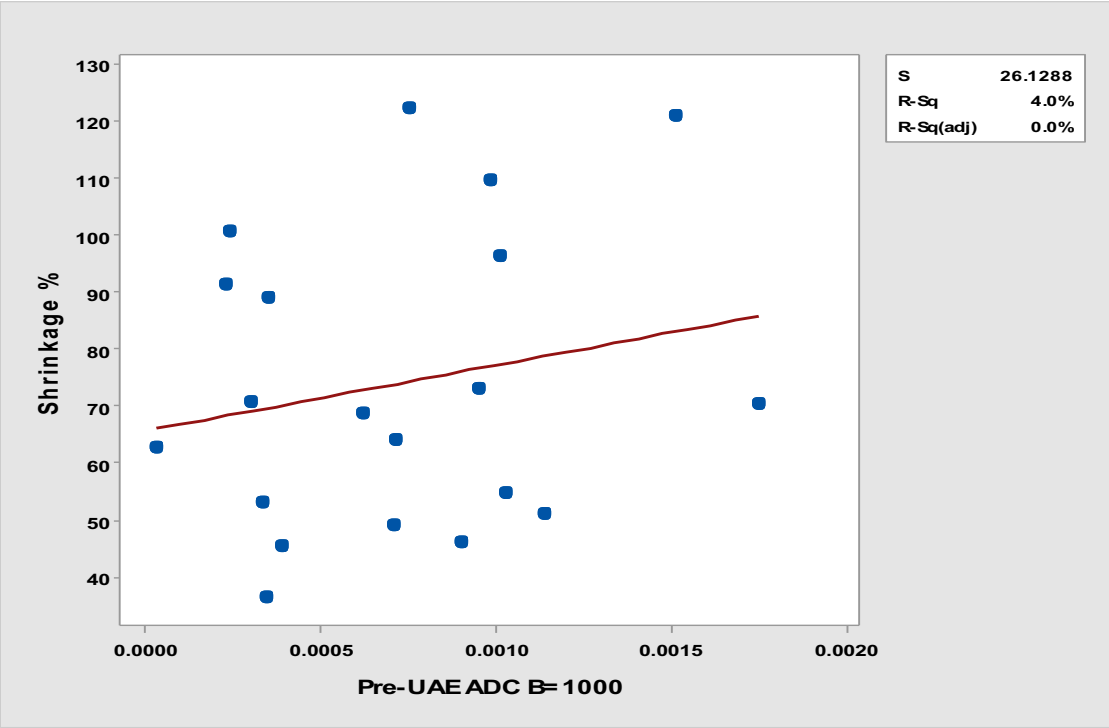


Figure 5-12 Line plot showing no correlation between pre-UAE ADC value of dominant fibroid and uterine shrinkage

We therefore conclude that the pre-UAE ADC value of the dominant fibroid explains much more of the variability in dominant fibroid volume reduction than it does for dominant fibroid shrinkage, uterine volume reduction and uterine volume shrinkage. This result is probably because we are only assessing the largest fibroid rather than all fibroids in a multi-fibroid uterus.

5.12 The Uterine components (Myometrium and Endometrium):

In this study a DWMRI was performed at Pre-UAE, 1 day post UAE and 6 months post UAE. This gives a unique insight into the changes that may occur at the level of the myometrium

and the endometrium immediately after UAE. By calculating the ADC value of these layers at the three different time intervals above, we attempted to detect when the cellular damage occurs. Assessing the changes to both the myometrium and the endometrium will aid in assessing UAE safety especially in those women that would like to keep their fertility. The ADC calculations in this section were performed at the highest level of sensitivity for this study (b=1000).

5.12.1 The Myometrium:

The ADC value of the myometrium was calculated by selecting a sufficient ROI that was separate from the endometrium or an existing fibroid. The same area was selected again on day 1 post-UAE DWMRI images and 6 months post-UAE DWMRI images. The ADC value was calculated at the highest sensitivity (b=1000).

5.12.1.1 Difference in ADC value of the myometrium between pre-UAE and day 1

We calculated the ADC value of the myometrium pre-UAE and day one post UAE in 11 patients (55% of the cohort). In nine patients we were unable to obtain both pre-UAE & one day post-UAE ADC values. As the distribution was non-parametric but paired we used Wilcoxon signed-rank test and found the difference was non-significant (table 5-9).

Table 5-9: The difference in myometrial ADC values pre and 1 day post UAE

	Myometrium ADC pre-UAE (n= 11)	Myometrium ADC day 1 post-UAE (n=11)	P value
Median	0.0093	0.00104	> 0.05

5.12.1.2 Difference in ADC value of the myometrium between pre-UAE and 6 months post UAE

Paired ADC values were calculated in 15 patients (75% of the cohort). In five patients we were unable to obtain both pre-UAE & 6 months post-UAE ADC values. We used a Wilcoxon signed-rank test and found the difference was non-significant (table 5-10).

Table 5-10: The difference in myometrial ADC values pre and 6 months post UAE

	Myometrium ADC pre-UAE (n= 15)	Myometrium ADC 6 months post-UAE	P value (95% CI)

		(n=15)	
Median	0.00107	0.00132	>0.05

The above results indicate that the ADC value of the myometrium is neither changed 24 hours post UAE nor at 6 months post UAE compared to the pre-UAE values. As the ADC value reflects the integrity of the cell membrane, therefore, no significant change to its value signifies no myometrial damage secondary to UAE. We conclude that despite the main blood supply of the uterus is blocked with UAE, the myometrium is not affected.

5.12.1 The Endometrium:

The ADC value of the endometrium was calculated by selecting a ROI on the endometrium on a DWMRI images with the widest section of endometrium. Care was taken not to include any myometrium or existing fibroid tissue. This was achieved by magnifying the endometrium to x 5 magnification. At the same time, the endometrial thickness was measured in mm. The measurements were performed on the pre-UAE DWMRI and repeated on the day 1 post-UAE DWMRI images and the 6 months post-UAE DWMRI images. The ADC value was calculated at the highest diffusion sensitivity (b=1000).

5.12.1.1 Difference in ADC value of the endometrium between pre-UAE and day 1 post UAE

Paired ADC values were obtained in 10 patients (50% of the cohort). A Wilcoxon Signed Rank test was performed. The difference in ADC values before UAE and at day 1 post UAE was not significant (table 5-11). This suggests no disruption to endometrial cellular membrane integrity in the immediate period after UAE.

Table 5-11: The difference in endometrial ADC values pre and 1 day post UAE

	Endometrium ADC pre-UAE (n= 10)	Endometrium ADC day 1 post-UAE (n=10)	P value (95% CI)
Median	0.00114	0.00142	P >0.05

5.12.1.2 Difference in ADC value of the endometrium between pre-UAE and 6 months post UAE

Paired ADC values were calculated in 14 patients (70% of the cohort). A Wilcoxon Signed Rank test was performed which showed no significant difference in ADC value of the endometrium pre-UAE and 6 months post UAE (table 5-12).

Table 5-12: The difference in endometrial ADC values pre and 6 months post UAE

	Endometrium ADC pre-UAE (n= 14)	Endometrium ADC 6 months post-UAE (n=14)	P value (95% CI)
Median	0.0011	0.0013	P > 0.05

This above analysis suggests no change to cellular integrity at 24 hours & 6 months post UAE compared to pre-UAE ADC values. This suggests that the cellular structure of the endometrium remains intact post UAE.

5.12.1.3 Endometrial thickness:

We could not control for when the 6 months post UAE MRI was performed in relation to the menstrual cycle. Despite this we felt that comparing the endometrial thickness post-UAE with the pre-UAE thickness is of value. We did not feel that a 24 hour comparison was meaningful and only compared pre-UAE to 6 months post UAE. The mean endometrial thickness post UAE was 4.46mm. This is encouraging as not only was the endometrium measurable but it was of similar value to the pre-UAE mean 4.36mm. We were able to calculate the difference in endometrial thickness in 18 patients (90% of the group). As the endometrial thickness was normally distributed we used a paired t-test and found no significant difference in the endometrial thickness between pre and 6 months post-UAE (table 5-13 & fig 5-13).

Table 5-13: Paired t-test showing the change in endometrial thickness

	Endometrium Thickness (mm) pre-	Endometrium Thickness (mm) 6 months post-	P value

	UAE ± STD (n= 18)	UAE ± STD (n=18)	
Mean	4.36 ± 1.9	4.46 ± 2.2	>0.05

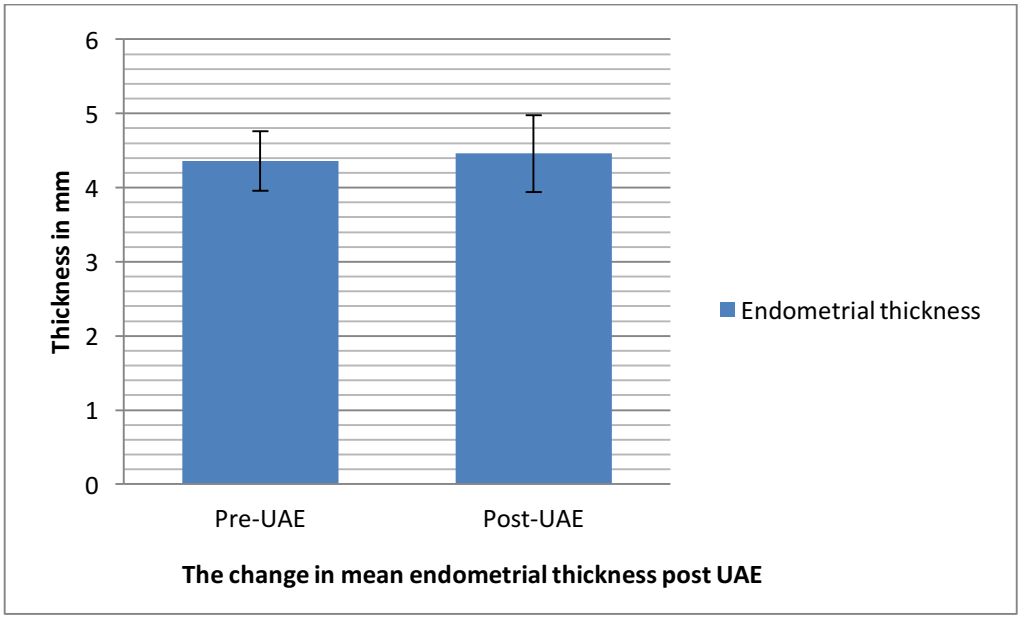


Figure 5-13: The mean endometrial thickness pre and post UAE

5.13 Discussion:

Diffusion weighted MRI is a relatively new clinical method of assessing uterine fibroids. It has been shown to aid in to distinguishing between a benign fibroid (leiomyoma) and a smooth muscle tumour of the uterus (leiomyosarcomas). This can be achieved by calculating the ADC value of uterine tumours and comparing it to a reference range [95]. Although further studies are needed to further validate this.

Fibroids are composed of densely packed smooth muscle cells with varying amount of intervening collagen. Prior to embolisation, these clusters of cells with intact cell membranes result in a restricted pattern of diffusion of water between the cells. Following UAE there is infarction of the fibroid and loss of the cellular architecture. This can be quantified by measuring the ADC value on MRI images with diffusion weighted sequences. In this study, our aim was to assess the relationship between the ADC value of a fibroid and its likelihood to respond to uterine artery embolisation. Through DWMRI and ADC measurement we also looked at the changes that occur in the different layers of the uterus in response to UAE.

This study was a prospective pilot study and benefited from a strict imaging protocol that was replicated for each patient. It did however suffer from a lack of power to detect true correlations between pairs of variables. However, it does pave the way for further research in the field of DWMRI especially in gynaecology. The study benefited from the use of a 3 Tesla (3T) MRI machine that is able to produce high resolution images. However, this machine was not capable of taking Diffusion weighted images at the same time as standard T1 & T2 weighted images. This resulted in minor movement artefacts that resulted in the inability of measuring ADC in a few of the subjects. Newer machines can compensate for this. Future software may be able to show the ADC map on top of the T1 & T2 weighted images (overlap) rather than in separate windows.

The aim of this study was to assess the changes in ADC value of the different components of the fibroid uterus and how this value changed after UAE. We wanted to compare the ADC value at baseline of the dominant fibroid, the myometrium and the endometrium to the value 24 hours post-UAE and 6 months post-UAE. As ADC values can reflect cellular damage, we intended to evaluate if the uterine components other than the target fibroid are

altered. A secondary aim was to assess if ADC could predict uterine fibroid shrinkage. This would then be used to predict the outcome of UAE prior to the intended procedure.

We found a significant reduction in the ADC value of the dominant fibroid between baseline (pre-UAE) and 6 months post embolisation at each level of sensitivity in this study. This reduction in ADC value suggests that cellular integrity is disrupted, causing cellular dehydration and cell death. This is evident in lack of contrast enhancement of fibroids that clinicians class as a "dead" fibroid and is used as a marker of success of UAE. Such marker can be clearly seen on a post UAE contrast enhanced MRI. Due to my late involvement in this study, I was unable to influence the imaging protocols as the DWMRI was performed in a transverse (coronal) plane which made it more difficult to obtain ADC values than if the sequences were obtained in a sagittal plane. This has been fed back to the respective MRI departments and a change of MRI protocol is being discussed for future research.

A moderately positive and statistically significant correlation was found between baseline ADC of the dominant fibroid (pre-UAE) and the volume reduction of that fibroid 6 months after UAE. This correlation was significant for each level of sensitivity (b= 250, 500, 1000). This suggests that knowing the ADC value of the dominant fibroid prior to UAE may be an indicator of how much loss in volume will occur post UAE. This may be individualised for each specific fibroid and is probably why no correlation was found when % shrinkage was used that doesn't take into account the volume of the fibroid. We were unable to find a significant correlation between the dominant fibroid and either the uterine volume reduction or uterine shrinkage post UAE. This may because a fibroid uterus does not necessarily have only one fibroid.

The aim of uterine artery embolisation is to treat fibroids and fibroid related symptoms. A successful UAE procedure is one that achieves this goal with no damage to surrounding uterine tissue (myometrium and endometrium). The ADC calculations for the myometrium and endometrium were based only on b = 1000 values as it was felt that there may be perfusion-related discrepancies in images acquired at lower b-values. The signal-to-noise ratio in the b = 1000 images were adequate for ADC calculation. However, it was not possible to measure the required ADC values for all the twenty study participants at this level of sensitivity due to image degradation. This will continue to be a challenge until image overlapping software is developed and DWMRI can be captured at the same time as

routine sequences. The later has become a reality but is only available in specialised centres.

It was not possible to measure the ADC value of the myometrium in each participant in this study due to difficulty in locating an area of myometrium that would not overlap with an area of fibroid tissue or endometrium. We compared pre-UAE myometrial ADC with Day 1 post UAE Myometrial ADC and found no significant difference. This indicates that myometrial cells may have regenerated after damage within the first 24 with acute pain being an indicator of damage. The other more likely explanation is that no damage occurred to the myometrial cells due to collateral supply and therefore the ADC value was unchanged. We arrived at a similar result that no difference was found when we compared the 6 months post UAE myometrial ADC value to that of pre-UAE. A previous study had unexpectedly found that the mean ADC value of the myometrium following embolisation was slightly reduced after UAE. The author suggests that it may have represented a technical problem rather than reflected a degree of myometrial damage [303]. However, another study looking at the perfusion of myometrium after UAE found a similar result to our study with no change in myometrial ADC. It too suggested that this was likely due to the development of collateral circulation supplying the myometrium [241].

The endometrium is vital for reproduction and the effect UAE has on the endometrium has not been studied well. In chapter 3 we looked at this in detail from a histological point of view. However, from an imaging perspective, specifically DWMRI, we compared endometrial ADC values pre-UAE to those 6 months post UAE. Although we had some difficulty in distinguishing the endometrium from surrounding myometrium at high sensitivity b=1000, meaningful analysis was performed. We found no statistically significant difference in the mean ADC value of the endometrium 24 hours post-UAE & 6 months post UAE to the mean ADC value of the endometrium pre-UAE. This indicates no change to cellular membrane integrity post UAE and suggests no damage has occurred. The endometrial thickness was also used as a crude marker of endometrial function. We found no significant difference between endometrial thickness at baseline and that at 6 months post-UAE ($p=0.92$). This supports ongoing normal endometrial function after UAE. This may aid women who want to preserve their fertility and are undecided regarding this treatment modality.

Research using DWMRI may help pave the way to prove that UAE is a safe treatment modality for fibroids and its effect could potentially be predicted for each individual patient. It may well prove to be a way of determining successful UAE procedures without the need for contrast enhancement. It may also give insight into the exact timing that the different components of the uterus are exposed to the hypoxia-like conditions associated with UAE.

6. Chapter 6

The role of Pictorial Menstrual Blood Loss Charts (PBAC) in assessing the success of uterine artery embolisation (UAE).

6.1 Blood loss estimation and symptom relief:

Heavy menstrual bleeding (HMB) is one of the most common complaints that women present to gynaecological services with [304]. It is defined as excessive menstrual blood loss which interferes with a woman's physical, social, emotional and/or material quality of life, however, this is subjective [37]. A clinician's usual practice is to enquire about the length of the period, days of heavy bleeding, presence of clots and flooding to subjectively diagnose HMB. However, such enquires have been shown to have no correlation with the severity of the complaint [305]. Objectively it is defined as more than 80ml during the period, although this definition is used for research purposes only [306].

There is a great deal of confusion regarding the terminologies used for abnormalities of menstrual blood loss. HMB is now a preferred description compared to menorrhagia or excessive per vaginal loss as it is easily understood and easily translatable [307].

HMB causes 1 in 20 women of reproductive age to consult their GPs and it accounts for 20% of all referrals to the gynaecology outpatients department. The quality of life is negatively affected and there is significant distress amongst women who are plagued with this symptom [308]. Clinically HMB can cause anaemia which can further exacerbate the physical symptoms [309].

Fibroids mainly cause HMB but can also give rise to pelvic pain, pelvic pressure and dysmenorrhea. Reproductive dysfunction and infertility may also be the main presenting symptom of fibroids [310].

6.2 The methods of determining menstrual blood loss:

6.2.1 Alkaline Hematin Method

Originally described by Hallberg and Nilsson in 1964 the Alkaline Hematin method is considered the gold standard method for menstrual blood loss quantification [311]. Measurement of menstrual blood loss involves extraction of haem from used sanitary towels using 5% sodium hydroxide, (about 100ml/ sanitary product). This is followed by incubation of the sanitary products in 5% sodium hydroxide solution for 48 hours to allow conversion of haemoglobin to haem. During the same time period a stored sample of the patient's venous blood should be used to create a 1:200 dilution of blood with 5% sodium hydroxide and an aliquot should be stored alongside the menstrual blood collection. After 48 hours, an aliquot from the mixture of sanitary products and 5% sodium hydroxide is obtained, filtered and diluted by measured addition of further sodium hydroxide. This then creates a close colour match with the control venous blood solution. The optical density (OD) of menstrual blood loss solution and venous blood sample are then measured using spectrophotometry. The menstrual blood loss is then calculated as a proportion of the patient's own venous blood using the following equation [312].

$$\text{MBL} = \frac{(\text{OD of Menstrual Blood Solution X Total Volume of added NaOH})}{\text{OD Venous Blood x 200}}$$

6.2.2 Pictorial Blood assessment chart (PBAC)

Higham et al introduced the Pictorial Blood Assessment chart (PBAC) in 1990 as an accurate semi-quantitative method for measuring menstrual blood loss (MBL) [168]. This method takes into account the total number of towels and tampons and the degree to which each item of sanitary protection is soiled with blood for a given menstrual cycle. This is essential as women use a varying number of towels and tampons to collect a similar amount of menstrual blood loss and no correlation between the measured menstrual blood loss and the number of towels and tampons used has been found. The number of sanitary products used in any given cycle is highly dependent on the socio-economic status and the hygienic needs of each individual [77]. A pictorial chart score of 100 or more, when used as a

diagnostic test for HMB, was found to have a specificity and sensitivity of greater than 80% [168]. While *Deeny et al* reported the chart as useful with 88% sensitivity and 52% specificity [313].

A number of studies set out to validate the use of the PBAC against the alkaline haematin method. *Zakcherah et al* concluded that the PBAC score is a semi-objective, simple and accurate tool to measure MBL and it can be used in clinical practice to help in managing women suffering from menstrual disorders. [314]. PBAC use has also been validated in women suffering from HMB secondary to fibroids [315]. In such cases the positive predictive value of the PBAC to distinguish women with HMB against the Alkaline Hematin method total was 91%. Measuring blood loss using PBAC is not without its problems as there is high inter-individual variation in its use but on the other hand has low intra-individual variation. However, from a clinical point of view a low PBAC scores or decreasing scores can be used to determine satisfactory treatment end points [316].

6.2.3 **Other methods of blood assessment**

Estimation of menstrual blood loss volume based on menstrual diary, laboratory parameters and age has been tried in women suffering from HMB. This was found to correlate well with the Alkaline Hematin method & could serve as an alternative method in estimating blood loss. [317] . However, simple methods such as counting and weighing sanitary products or filling menstrual diaries, only provide qualitative data on blood loss which reduces the value they have in research studies [318].

6.3 The aim of this chapter

Menstrual blood loss was estimated in patients undergoing UAE as part of the UAE study (chapter3) using the pictorial blood loss chart. Each study participant was shown how to fill in the PBAC diary prospectively for one menstrual period prior to UAE and one menstrual period 6 months post UAE. This was used to show the effect UAE had on reducing HMB associated with fibroids. In addition to this, we wished to determine if there was any correlation between the change in MBL and the endometrial markers that were studied in chapter 3 and the MRI-measurable changes in chapter 4.

6.4 Materials and methods

The participants in the histological studies were given a PBAC diary to fill in prospectively for one period prior to the UAE procedure (fig 2-6 & 6-1). The scoring method used to evaluate the PBAC score of the diary was not revealed to the patients. A further PBAC diary was sent to the patients to fill in by post 6 months from the day of UAE. The PBAC diary consists of a series of diagrams representing lightly, moderately and heavily soiled tampons or towels. The participant would mark the appropriate box at the time that each sanitary product was discarded. In addition, passage of clots and episodes of flooding were recorded. (Section 2.5.6)

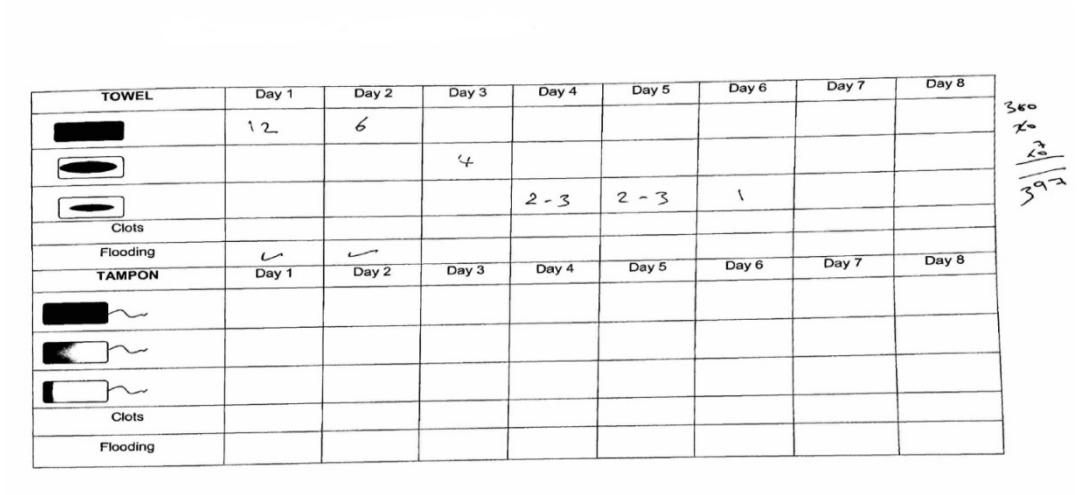


Figure 6-1: Completed PBAC for one menstrual cycle

Table 6-1: Illustrates an example of calculating a PBAC score from the PBAC chart above.

18 Heavily soaked towels	18 x 20
4 Moderately soaked towels	4 x 5
7 Lightly stained towels	7 X 1
Flooding	2 X 5
Total score =	397

6.5 Statistical analysis

We used a paired t-testing, Mann-Whitney test and one-way ANNOVA for statistical analysis to look at the changes in PBAC score, length of cycle and number of sanitary towels used pre and post UAE. Parametric (Pearson's) and non-parametric (Wilcoxon-sign-rank) correlation tests were used to analyse the correlation between the changes in PBAC score and the changes in endometrial markers and MRI.

6.6 Results:

UAE is a treatment for HMB in women with uterine fibroids and therefore a reduction in PBAC score, period length & heaviness is expected after treatment. Sixteen women were included in this study that filled both a pre-UAE and a 6 months post-UAE PBAC diary. The mean age was 45.4 years (range 36-52).

6.6.1 **The change in PBAC Score**

The participants PBAC scores pre-UAE ranged from 62 to 1419 (Mean =490, Median 260). The majority of individuals (15/16, 93.7%) had a pre-UAE PBAC score of >100 suggesting HMB. After treatment with UAE the number of individuals with PBAC > 100 significantly reduced (5/16, 31.2%). We found that the reduction in PBAC score 6 months post-UAE compared to pre-UAE was significant (P < 005). This translates to an overall reduction in HMB for this group of patients.

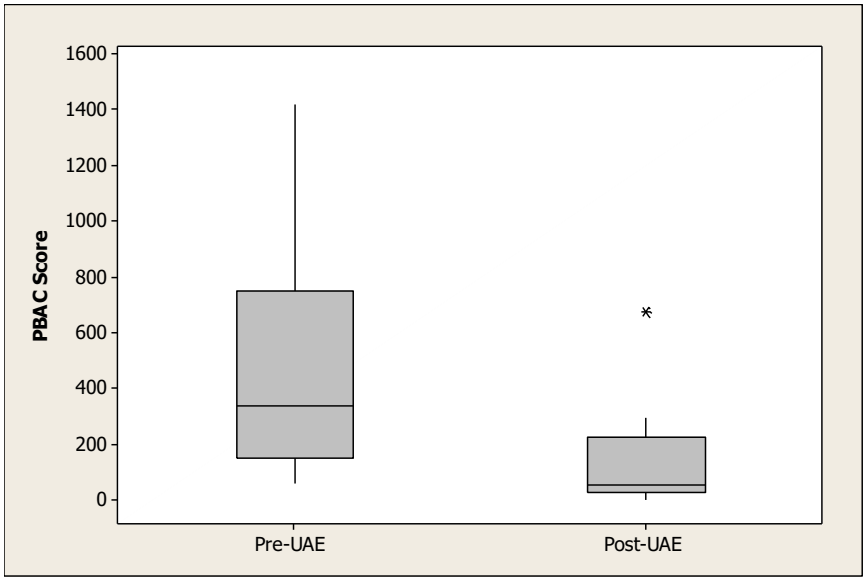


Figure 6-2 Box plot showing the change in PBAC score

We also looked at the correlation between pre-UAE PBAC score and how much the score reduced after UAE using Spearman Rank test. We found this to be a significantly strong positive relationship (R-sq= 85.8%, ρ = 0.902, P-value < 0.001). This means that the higher the PBAC score the more it will reduce after treatment. Clinically this shows that women who are suffering from severe forms of HMB may benefit the most from UAE.

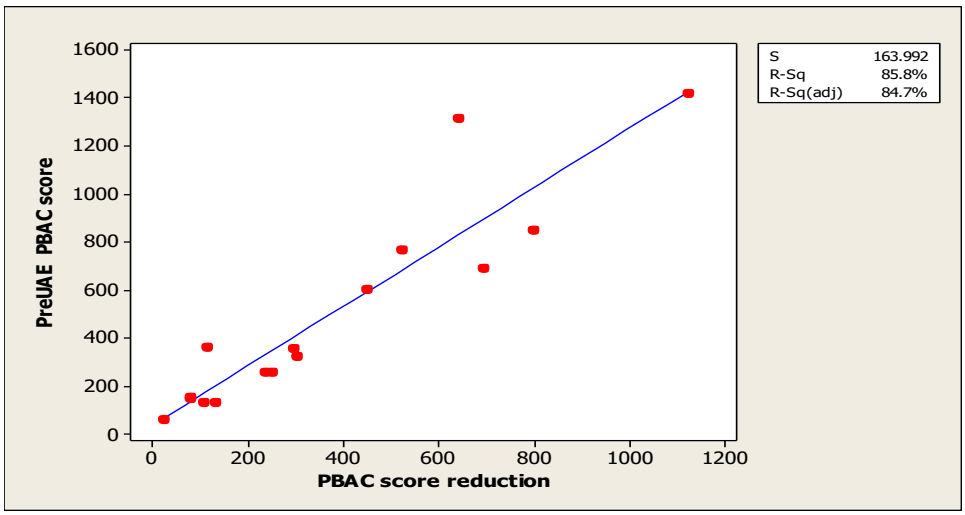


Figure 6-3 Correlation of Pre-UAE PBAC score vs PBAC score reduction

6.6.2 **The change in sanitary product use**

Each woman used an average of 31.5 sanitary products throughout their period. There was an unsurprisingly strong positive and significant correlation between the PBAC score pre-UAE and the number of sanitary products used (R-Sq = 73.1%, , ρ = 0.87, P < 0.001) (fig 6-3). *Higham et al* found a similar positive correlation between the total number of sanitary products used and the menstrual blood loss in ml. [168]. We could not find a correlation between the pre-UAE PBAC score and the period length. It has been shown that during most cycles women report the heaviest bleeding on the second day of menstruation [319].

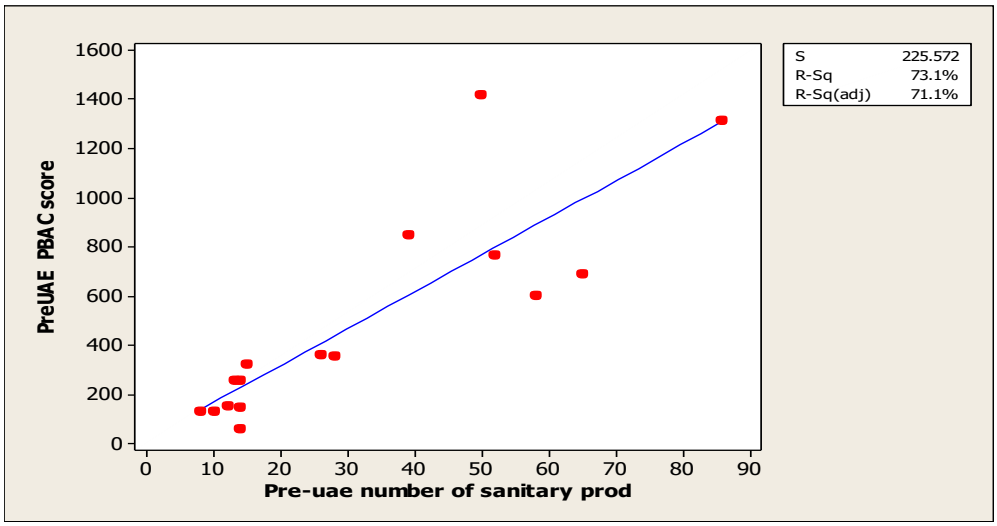


Figure 6-4: Correlation of Pre-UAE PBAC Score & number of sanitary products used.

We found a significant reduction in sanitary product use (tampons and towels) post- UAE treatment (P = 0.01) (Fig 6-5).

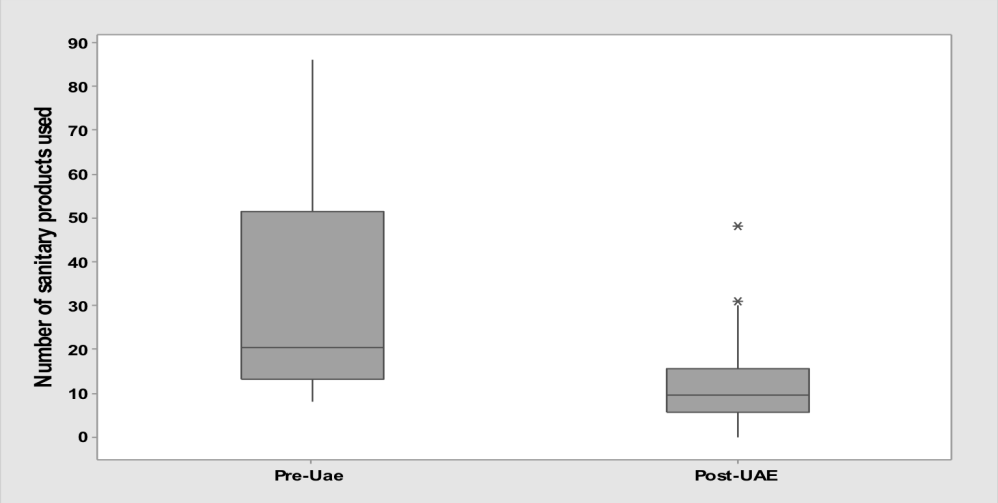


Figure 6-5: Bar chart showing the change in sanitary product use

6.6.3 **The change in period length**

The length of the period varies from one woman to another. The prolonged duration of a period for a woman with a fibroid uterus directly impacts on the quality of life. However, this did not appear to correlate to the PBAC score. We found the period length was significantly shorter after treatment with UAE (p=0.002) (Fig 6-6).

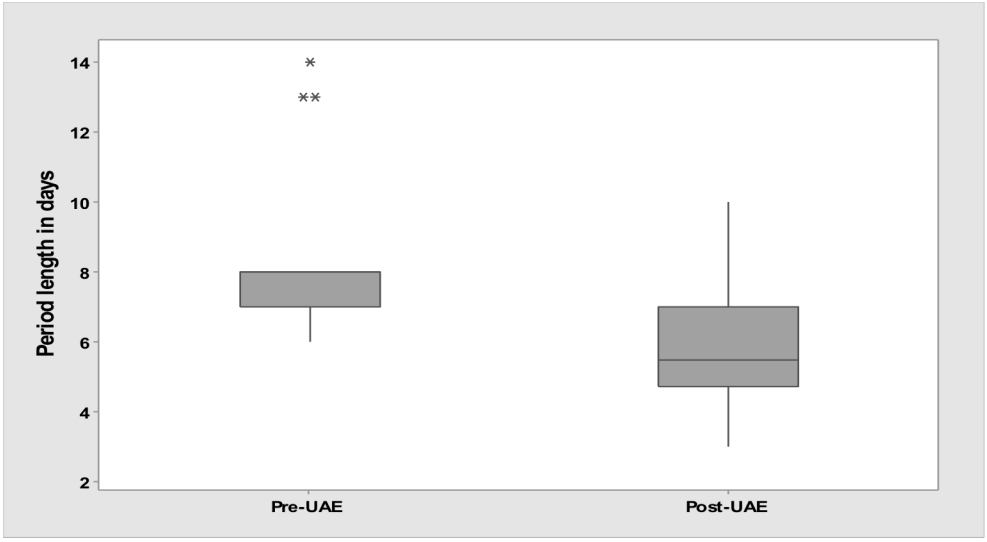


Figure 6-6 Bar chart showing the change in average period length

6.6.4 The uterus and the dominant fibroid

To test if there are significant differences in PBAC score depending on the dominant fibroid location we ran a one–way ANOVA test. The results showed that there are no significant differences in PBAC score according to the location of the dominant fibroid. This was probably due to not enough variation in the data as the majority of patients in this group had dominant fibroids that were intramural.

We could not find any relationship between pre-UAE PBAC score and any of the following: pre-UAE fibroid size, pre-UAE uterine size, fibroid shrinkage, uterine shrinkage or age of the patient at the time of UAE.

6.7 Correlation between PBAC score & studied endometrial markers

We looked at the correlation between the PBAC score and the expression of the different markers that we measured in the endometrium in chapter 3 both pre-UAE & post UAE. We found no significant correlation between them.

Table 6-2: Showing the correlation between PBAC and endometrial marker expression

Endometrial Marker	Spearman Rho Correlation to Pre-UAE PBAC Score	P =		Spearman Rho correlation to Post-UAE PBAC score	P =
VEGF-A	-0.34	0.30		0.44	0.17
COX-2	-0.27	0.41		-0.17	0.59
MVD	0.14	0.67		0.33	0.31
Ki67	0.38	0.24		0.5	0.09

We also looked at the change in PBAC score compared to the change in the endometrial markers expression in the endometrium and found no significant correlation between them.

Table 6-3: Showing the correlation between the change in PBAC and the change in marker expression/measurement

Endometrial Marker	Spearman Rho correlation between change in PBAC score to the change in endometrial marker post-UAE	P =
VEGF-A	-0.63	0.12
COX-2	0.07	0.21
MVD	0.46	0.29
Ki67	0.02	0.95

6.8 Discussion

Although the pictorial blood loss assessment chart is a subjective tool for estimating menstrual blood loss, it has been proven to be reliable. It has been validated against the objective estimation method of measuring blood loss (alkaline haematin method) [315]. Modern sanitary pads may impact on the validity of PBAC scores as they convert liquid to non-evaporating gel, therefore underestimation of blood loss may occur [320].

In this study instructions were given to the participants on how fill in the PBAC chart prospectively and no instruction on the method of calculating the PBAC score were given.

Although some authors point out that about 50% of women who complain of heavy menstrual bleeding actually have normal menstrual bloods loss, in this study only one woman (6% of participants) had a PBAC score < 100. There is an obvious monthly menstrual bleeding variation and the use of medicines to alleviate heavy menstrual bleeding was not accounted for in this chapter.

The median PBAC score in this study was high (340). This was 3 folds higher than the median score for Higham's population in 1990. This may be explained by the fact that our study included women with fibroids will Higham's study was of normally menstruating women [321]. Two PBAC scores in this study were ≥ 1000, which corresponds to approximately a blood loss of ≥750ml. This is a large amount of blood loss which on a recurrent monthly basis would render an individual severely anaemic. Even when these two patients were excluded from analysis, the results did not change.

Some women may use small size and/or low absorbency sanitary materials and a direct comparison could not be made as most participants did not fill in the brand name used. A patients' personal hygiene and their perception of the degree of soiling of a tampon or towels could also be an explanation for these high PBAC scores.

The median PBAC score dropped significantly after UAE (363 to 85) and this is an encouraging finding. Most women who attend for UAE were seeking a reduction in menstrual blood loss and this result clearly describes this. As the PBAC score dropped, so did the length of the periods and the need for sanitary product use. Such an effect is

expected as these variables are directly linked to one another. Although, no quality of life questionnaires were used, the result from this study point towards an improvement in the quality of life of women whom were treated with UAE.

We found a positive correlation between pre-UAE PBAC score and the amount it drops after UAE. This suggests that women who are suffering from severe forms of HMB and have very high PBAC scores are likely to benefit the most from UAE.
We could not find a correlation between the pre-UAE PBAC score and the period length. Suggesting that although women with uterine fibroids have heavy periods they don't necessarily have lengthier ones. We mentioned earlier in the chapter that the heaviest blood loss of a period is usually on day 2.

The size of the fibroid uterus and the dominant fibroid pre-UAE did not correlate to the PBAC score. Clinically this is usually the case as many women with small fibroids present with HMB and others with large fibroids may be found incidentally or present with other symptoms like pressure.

We found no significant correlation between the expression of markers studied in pre & post-UAE samples to their respective PBAC score. This suggests that a dramatic drop in PBAC score does not significantly change the markers studied. Another explanation is that clinical improvement does not reflect the changes in the endometrium or simply the number of samples were too small.

The pictorial blood loss assessment chart is a semi-objective method for estimating blood loss. It is easy to understand and most patients find filling it non-taxing. It can be used to evaluate the effectiveness of certain treatments for heavy menstrual bleeding such as UAE. This is done by showing the difference in PBAC score before and after treatment. However, its use in women with fibroids and those with very heavy bleeding might be limited and may not reflect the true blood loss. There is currently an ongoing study (FEMME Trial) that is collecting data from over 250 patients and comparing the pre-UAE PBAC score to those 6 months and 12 months post UAE. It may be that a different conclusion can be reached with more samples.

7. Discussion

7.1 General Discussion

Uterine fibroids are the most common benign tumour affecting women of reproductive age. They are widely prevalent and up to 50% of women suffering with fibroids are symptomatic [55]. Women seeking treatment for fibroids account for up to 20% of the gynaecology outpatient referrals [39]. Although a range of treatment options are available they mainly fall into two categories, medical and surgical treatments. Although well tolerated and relatively easy to administer, medical treatments until recently have only helped in symptom control. Apart from GnRH analogues, SPRMs seem to be a successful; non-invasive treatment that is aimed at both symptom control and fibroid size reduction. Long-term trials are in place assessing this group of drugs [125]. Surgical options such as hysterectomy and myomectomy have been the mainstream method of tackling fibroids. The advances in interventional radiology and the emergence of UAE in 1995, have given women a less invasive option that targets both symptoms and size of the fibroid uterus [132].

Although UAE is now a well-established method of treating women with fibroids, the exact mechanism of how it works and which patient achieves maximum benefit is not fully clear. However, it is clear that it acts on multiple layers within the fibroid uterus. HMB reduces after treatment, signalling a change in the endometrium's response to the cyclical hormonal changes that affect it on a near monthly basis. The fibroid uterus generally and the fibroids specifically reduce in size pointing towards an effect on the myometrium. Reduction in subserosal fibroids proportionately reduces the surface area of the serosa as well. While shrinkage and expulsion of submucosal fibroids directly impacts on the function and regularity of the endometrial cavity which in turn may have a positive impact on fertility [83]. Our aim in this thesis was to explore the mechanisms by which UAE affects the endometrium and the impact of this procedure on the fibroid uterus.

There is evidence that endometrial factors such as VEGF and the prostanoids /cyclooxygenase system may be involved in the control of menstrual blood loss. VEGF plays a role in restoring blood supply through angiogenesis to the endometrium after menstruation [185]. It also plays a critical role in angiogenesis of tumours such as fibroids. COX enzymes have been found to have a role in many reproductive tract pathologies such as HMB which is studied in this thesis [191]. Therefore studying the expression of VEGF-A &

COX-2 in the endometrium before and after UAE may explain the mechanism by which it affects this layer of the uterus.

MVD is understudied in the endometrium and has never been studied in the endometrium of women after UAE. There has been one study that showed no correlation between VEGF & MVD in the endometrium of women with adenomyosis but not of women with uterine fibroids [204]. The aim was to assess the assess MVD across the menstrual cycle on women with fibroids and its rate of change following UAE.

Ki67 as a marker of proliferation and can be used to evaluate the growth fraction in any human cell population. It is absent from resting cells that are not dividing. The endometrium in women with endometrial hyperplasia who often present with irregular and heavy bleeding, has been shown to have increased proliferation and therefore increased expression of Ki67 [212]. Hence a treatment modality that reduces menstrual blood loss such as UAE, may lead to detectable change in the expression of Ki67.

Women of different age groups present to the interventional radiology clinic requesting UAE. Some studies found age not to be an important factor in determining UAE success as it did not correlate to the change in size of the fibroids while others found increased age predicts failure of UAE [250, 252]. The number, size and location of fibroids at the time of presentation all seem to play a role in determining the outcome. MRI is best placed at objectively assessing these factors both before and after UAE. As with any intervention, UAE can fail and is not without risks and complications. The effect UAE has on a woman's ability to reproduce is still not fully understood. Despite the importance of such an effect, it remains understudied because of the large numbers needed for any meaningful trial to be undertaken. This can mainly be attributed to the active exclusion of women wanting to retain their fertility from studies involving UAE [148, 265]. The results of the FEMME trial which did not have such exclusion are eagerly awaited [322]. Currently, pregnancy data following UAE is derived from case series rather than randomised controlled trials [323]. DWMRI has the ability to detect disruption to cellular membrane integrity through quantifying the diffusion of water between cells by objectively measuring the ADC value of the studied cell population. Using this MRI sequence before and after UAE can detect changes in specific regions of interest in the fibroid uterus. These changes can shed light on

what component of the uterus is affected by UAE and potentially its mechanism of action. ADC values are reproducible values and may form part of a standard MRI report in the future as it can quantify overall cell membrane disruption and cellular damage.

The limitations of the endometrial sample study:
1-It lacked power; this was mainly due to difficulty in recruiting as the larger FEMME trial was ongoing at the time. Endometrial sampling using a Pipelle™, although tolerated as a necessity, can become a laborious process for women if performed in the context of a study and some women who have previously experienced endometrial sampling refused to participate.
2-Statistical analysis was not always possible between the groups compared as these numbers were small and only the trends were described.
3-The inability to obtain fibroid samples before and after UAE as this would require CT-guided biopsy which is not within the realm of this study.
4-Not all the endometrial samples obtained could be used for this study as some showed a chronic endometritis picture that would alter the expression of the studied markers.
5-Although the timing of the pre- and post-UAE were synchronised to coincide with the same phase of the menstrual cycle

The strengths of endometrial sample study:
1-It was a prospective study and we were able to look at four different markers in the endometrium despite the small amount of tissue obtained
2-It benefited from direct comparisons that were made between endometrial samples taken from the same group of patients both before and after UAE allowing for paired comparisons to be made.
3- The cost of this study was low as both pre & post-UAE MRI was part of the clinical assessment package for each patient. The researcher (myself) was a senior gynaecology registrar who is experienced in performing endometrial sampling and did not require training or supervision.

The study found VEGF-A expression tends to increase at 6 months post UAE signalling neovascularisation. The mean VEGF expression (H-Score) measured after IHC, increased in samples taken in the proliferative stage of the cycle. This stage of the cycle is immediately

after menstruation and involves regeneration of cells in the basilar zone of the endometrium. Such a process requires angiogenesis in which VEGF is essential [181]. It may be that the rise in VEGF helps in regulating the menstrual process and rearranging any abnormal physiology that caused HMB in the first instance [187]. Therefore a normal start to the regeneration process of the endometrium, then paves the way for the rest of the menstrual cycle to continue normally and improving HMB. This may explain why the changes in VEGF were more pronounced in the proliferative stage rather than the other stages of the cycle.

This thesis found a similar rise in VEGF in post-UAE samples when studying the relative expression of VEGF RT-PCR. The limits of this part of the study were mainly due to sample size and the two different extraction methods used. After the move of the Pathology department from Glasgow Royal Infirmary to the South side of the City, it was no longer possible to snap freeze samples. Therefore extraction was performed from paraffin samples as well as from fresh frozen samples already collected. It was difficult to perform meaningful comparisons after RT PCR due to small sample numbers. However, unpublished data by fellow researcher Salha Abukhnjr suggests reasonable agreement between paraffin and fresh frozen sample results. Statistically significant results were found when looking at the cohort of samples from normally menstruating women (active samples). Results were only obtained from one subject were both pre-UAE & post-UAE samples were taken in the proliferative stage. This however showed a large rise in VEGF RT-PCR post-UAE echoing the results obtained from the IHC analysis. Another explanation of such rise is that VEGF is usually low in the proliferative stage of women with HMB and the observed increase represents a return to normal. A large sample is needed to study the expression of VEGF in the endometrium of women with fibroids at different stages of the cycle.

This thesis found that COX-2 expression was mostly weak in the samples studied. This is in keeping with the fact that the highest levels of COX-2 are seen in menstruating samples and none of the samples studied were in that stage of the cycle [218]. Excessive expression of COX-2 has been found in the endometrium of women with HMB [25]. It may be that the observed downward trend of COX-2 expression post UAE points towards regulation of the inflammatory process post UAE. This in turn is similar to the effect seen with the use of NSAIDs such as mefenamic acid – a COX inhibitor -that has been shown to reduce HMB [41,

104]. It is perceived undignified by most women to be examined while menstruating. However, future research into menstruating samples could alleviate the difficulty in pairing samples to the same stage of the sample and increase the sample size significantly in a study like this.

This thesis also found a significant increase in MVD of the endometrium post UAE. This was evident in paired samples were direct comparison was made. MVD was found to rise in all samples from normally menstruating women and those with inactive samples. The uterine arteries are the main blood supply of the uterus and blocking these two vessels by UAE is an ischaemic event. This event leads to hypoxia-like conditions which are known to enhance the expression of VEGF and stimulate angiogenesis [15]. This should then be evident by an increase in MVD and such an effect was observed in the samples studied in this thesis.

We studied the expression of Ki67 as a marker for proliferation and found an increasing trend of proliferation in the proliferative stage and early secretory stage samples post-UAE. The sample size was small and statistical analysis was not possible. This increasing trend echoes the changes seen in VEGF-A & MVD. Such directly proportionate change albeit a decreasing one has been seen in treatment of prostate cancer in mice suggesting a direct interaction between these processes [324]. We found no change to Ki67 expression in late secretory stage and inactive samples. Although the level of expression of Ki67 has not been compared in the various stages of human endometrium. A study involving mice has shown varying levels of expression throughout the rodents' estrous cycle [215].

This study found a significant reduction in PBAC scores post-UAE. However, we found no correlation between the expression of the studied endometrial markers and PBAC scores pre-UAE nor the change in PBAC scores post-UAE. This is probably because PBAC charts are designed to assess the change in one clinical symptom -HMB- that is subjective in each woman. While endometrial markers are affected by the changes occurring in the fibroid tissue post-UAE. It may also be explained by how different women completed the PBAC chart and the subjective perception of a lightly or heavily soiled pad or tampon.

This thesis looked at a cohort of patients who had undergone UAE over a 5 year period. The study found that 95% of the patients had a reduction in the size of their uterus & dominant fibroid. The mean dominant fibroid volume reduction ranged between 38-70% which is similar to the levels previous studies have reported[325]. The fibroid uterus reduced in volume by around 40%. When reviewing a sample of the case notes, we found the overwhelming majority had HMB pre-UAE. Therefore a 4% re-intervention rate at 6 months and a 15% re-intervention rate at 18 months reflect a short-medium term success rate and suggest resolution of main referral symptom. Although the complication rate of UAE is low, re-intervention rate and the need for additional treatment appear to increase with time. This reverberates with what has been found in other studies that looked at long term outcomes post UAE [148, 265].

The limitations of the five year UAE practice study are:
1-It was a retrospective study and a large amount of follow up data and imaging was missing.
2-Contrast enhancement of the fibroids was not analyzed and therefore not used as a separate confounder when assessing the change that occurs to the fibroid studied. This was done due to a wide variation in the practice of measuring contrast enhancement and it being a subjective technique.
3-Only the dominant fibroid was measured rather than all fibroids and the reasoning behind this has been explained earlier in the thesis.
4-The measurements of the dominant fibroid and the uterine diameters were performed by one researcher however, adequate training was given beforehand.

The strengths of the five year UAE practice study are:
1-Despite data loss, a large number of patients had complete data and imaging.
2-The study benefited of the use of MRI for pre & post-UAE analysis which is the gold standard for imaging in fibroids.
3-No funding was required for image analysis as the images were already stored on PACS and were readily accessible.
4-The research was blinded to post-UAE images as all Pre-UAE images were analyzed first followed by post-UAE image analysis.

This study found that small or medium sized dominant fibroids (<700 cm^3) shrunk by 11% more than large dominant fibroids. This suggests that these fibroids are more susceptible to UAE and it may be that they are exposed to a smaller -by surface area- vascular bed than larger fibroids. This result was different to what *Czuczwar et al* who found, however, a different cut-off value for large fibroids and a different method of measuring fibroids was used [326]. We could not demonstrate a difference in shrinkage between a large uterus (≥780 cm^3) and a small uterus. This may be that the shrinkage of the uterus is reliant on the shrinkage of the different sized fibroids within it and cannot be used as a separate entity from the fibroids that are targeted. As we only looked at the dominant fibroids, analysis involving all fibroids within a uterus may yield a different outcome. Measuring and locating all fibroids in a multi-fibroid uterus can be a challenging process to even experienced radiologists.

The mean age of presentation for women who undergo UAE was 45y. This late presentation has directly impacted on UAE studies looking at fertility. Our study could not find a correlation between the age of the woman at the time of UAE and the size of the fibroid uterus or the dominant fibroid. We did however; find a positive correlation with shrinkage. Women above the age of 50y were found to have the best response from UAE. As the average age that women in the UK reach menopause is 51y, we suggest a synergistic shrinkage effect between a peri-menopause state and UAE. One explanation of such outcome is the decrease in ovarian function that occurs after UAE and is concentrated in women older than 45 years [135]. Such a decrease may be accelerated by the effect of UAE and directly impact on the fibroid shrinkage. We conclude from this that women close to the age of menopause should be counselled appropriately regarding opting for UAE rather than surgery especially if pressure is the main symptom. Our study found this group of women to have the best MRI-measurable shrinkage which theoretically should reflect better resolution of pressure symptoms.

The study also found that dominant fibroid shrinkage is related to its position. Submucosal fibroids shrunk more than intramural fibroids by 26.6% and more than subserosal fibroids by 31.8%. This may be explained by the method that was used to measure submucosal fibroids that were expelled (100% shrinkage). Another explanation is that submucosal fibroids are more sensitive to the effects of UAE because of the distribution of embolic particles, more of which settle in the inner part of the myometrium [326]. Although fibroid expulsion is

considered a complication of UAE, the removal of submucosal fibroids leads to a significant increase in pregnancy rates and a decrease in miscarriage rates [83]. It might be that submucosal fibroid expulsion should be counselled as a positive outcome in women contemplating pregnancy.

The study also found that women presenting with a single fibroid in the uterus had a larger uterine shrinkage compared to those with multiple fibroids. This may be that the limited volume of embolic particles used that initially causes uterine artery stasis is then distributed over a larger vascular bed in a multi-fibroid uterus after uterine artery recanalization. There are no studies that have looked at the volume of embolic particles used and the outcome of UAE as most studies have only looked at comparing the outcome of different embolic agents.

Through studying DWMRI this thesis used the ADC value to evaluate the changes to the cellular membrane structure of the cells forming the layers of the uterus.

The limitations of the DWI study are:
1-Lack of power but this was mainly to do with it being a pilot study
2-Analysis was performed by one observer as funding for a second researcher to perform a repeat analysis was not available. However, training in the use of DWMRI and the use of the software used for the analysis was undertaken.
3-Only the ADC of the dominant fibroid was used and not for every fibroid in a multi-fibroid uterus.
4-Image distortion at high b-values made ADC value calculations difficult and in some instances impossible.

The strengths of the DWI study are:
1-It was a prospective study and adequate funding was in place to support the researchers involved
2-The MRI sequences used were refined prior to using them in this study by researchers that work in this field.
3- Patients in the study underwent an MRI of the abdomen and pelvis on three separate occasions. The first and second MRI was done immediately pre and 24 hours post-UAE while the patient was an inpatient. This reduced dropout rates and helped to give a picture of

what happens in the immediate period after UAE. This period has rarely been studied before by MRI.

This thesis found a significant reduction in ADC value of the dominant fibroid post UAE. This is a similar result to that described by *Leapi et al* [327]. This suggests a disruption to the cellular membrane of the densely packed cells of the fibroid leading to cell death and fibroid necrosis and shrinkage. A statistically significant correlation was found between the volume reduction of the dominant fibroid and its ADC value prior to UAE. It may be that ADC can be used to predict the outcome of UAE for the dominant fibroid and other fibroids within the uterus. However, there was no correlation between dominant fibroid ADC value and the change in volume of the fibroid uterus. It may be that because a fibroid uterus contains different sized fibroids with different locations and that ADC of a specific fibroid only predicts that fibroids' change. This in turn gives ADC a limited prediction of change value. Further research measuring the ADC value of all fibroids within a fibroid uterus may be required. It may then produce a more specific shrinkage prediction model.

UAE is applied the uterus as a whole by blocking the two uterine arteries. Future advances in interventional radiology may see the rise of specific fibroid vessel targeting to avoid the unnecessary exposure of the whole uterus to the embolic agents used. Our study found that the ADC value of the myometrium was not changed in the first 24 hours post UAE nor was it changed 6 months post UAE. Despite finding no difference, this result signals the resilience of myometrial cells to the effect of UAE. It could also be explained by the specific targeting of the fibroid by the embolic particles used. Therefore, allowing an early end point to embolisation by settling in the fibroid tissue and minimalizing ischemic damage to the normal myometrium [328].

DWMRI is able to detect adverse changes to the endometrium. ADC change in the endometrium has been shown to identify malignant changes as there is no overlap between the ADC values of endometrial cancer and that of the normal endometrium [327]. Our study found no difference when comparing ADC of the endometrium pre-UAE to that at 24 hours and at 6 months post-UAE. This result is encouraging as despite reduction in HMB, the cellular integrity of the endometrium is unaffected. This can be interpreted as no sign of adverse effect found in the endometrium post-UAE.

A further encouraging result was that endometrial thickness was unchanged 6 months post UAE compared to baseline. Although this study did not specify/ sanction the timing of the MRI in relation to a woman's menstrual period, group analysis across the different stages of the menstrual cycle found no change to endometrial thickness. A directly proportionate relationship has previously been found between endometrial thickness and IVF pregnancy rates [329]. Therefore, the lack of change in average endometrial thickness in our study suggests no change to fertility but no improvement either.

7.2 Conclusion

- UAE caused an increasing trend in VEGF expression in the human endometrium which was more evident in the proliferate stage. It was also associated with a decreasing trend in COX-2

- Significant increase in endometrial MVD was evident post UAE.

- An increasing trend of Ki67 expression in post UAE samples was found. This suggests an interaction between VEGF, MVD & the proliferation of the endometrium.

- PBAC is a useful aid to assess blood loss in UAE however the scores do not correlate with the studied endometrial markers or the MRI changes.

- Women with a small and medium sized dominant fibroid or a single fibroid uterus and those approaching the age of menopause seem to gain the most benefit of UAE.

- The position of the fibroid does impact on its shrinkage and submucosal fibroids appear to be the most affected by UAE.

- DWMRI is a useful tool in assessing UAE outcomes and it shows that the different unaffected parts of the uterus remain unaffected by the ischemia-like conditions that UAE creates in the fibroid uterus.

7.3 Future work

This varied work that included a prospective laboratory study and retrospective & prospective clinical MRI studies has proven to be an interesting challenge. Although the aim was to identify the elusive mechanism by which uterine fibroids – a benign condition - cause heavy menstrual bleeding, the work that I have done will definitely have an impact on any future research in gynaecological cancers that I plan to undertake.

Regardless of the non-statistically significant results from comparing endometrial samples before and after UAE, the thesis supports further research into the role pre-UAE MRI has to play in the diagnosis and treatment of uterine fibroids. Previous unpublished work by my colleague Dr Salha Abu-Khnjr suggested up-regulation of the studied markers when comparing women with uterine fibroids to those without fibroids. These markers were used in this thesis to compare one specific treatment modality. It may be that this limited number of markers hold part of the answer to how UAE works and other markers may need to be studied on the samples obtained.

Future work with selective progesterone receptor modulators such as Esmya™ (Ulipristal Acetate) may give a better insight on how fibroids affect the uterus globally and the endometrium specifically. In practice these drugs cause fibroid tissue to soften and sometimes shrink therefore directly opposing the growth element in fibroids. They cause amenorrhea which is a desirable symptom in women with fibroids. SPRMs are still in their infant stages in regard to fibroid treatment. Progesterone Receptor Modulator Associated Endometrial Changes (PAEC) is a side effect that may limit their use and could potentially prove them to be hazardous.

Diffusion weighted MRI may hold answers to conditions and treatment modalities that damage cells. This thesis has shown that quantifying the changes after UAE can be accurately measurable. Our MRI unit has a large archive of pelvic MRI scans with the DWI sequences that have never been reported or analysed. Since the start of this thesis to date there has been considerable interest in DWI especially in gynaecology imaging and more radiologists are giving these sequences the attention they need. DWI MRI can sometimes help in differentiating leiomyosarcomas from leiomyomas and avoid treatment delay.

Future research in gynaecology oncology could utilize DWI MRI as both a screening tool and also to test new chemo-therapeutic agents. It has certainly been utilised more recently in the field of obstetrics in diagnosing abnormally invasive placental conditions such as placenta accreta.

Future plans are in place to analyse data from the FEMME trial and study the changes to Anti-müllerian hormone post UAE in 2017. I hope to continue to study the impact of UAE on the fibroid uterus through further research into DWI MRI. This imaging sequence may aid in both screening and treating benign and malignant gynaecological conditions.

Appendices

Appendix 1

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 5
Ground Floor - Tennent Building
Western Infirmary
38 Church Street
Glasgow
G11 6NT

Date 18 March 2015

Direct line 0141 211 2482
Fax 0141 211 1847
E-mail WoSREC5@ggc.scot.nhs.uk

Dr Ibraheem Hamoodi
Clinical Research Fellow in Gynaecology
New Lister Building Level 2, Room 2.56
Glasgow Royal Infirmary
10-16 Alexandra Parade
Glasgow
G31 2ER

Dear Dr Hamoodi

Study title: **Uterine Artery Embolisation : is it an acceptable treatment and why does it work ?**
REC reference: **10/S1001/1**
EudraCT number: **N/A**
Amendment number: **2**
Amendment date: **11 March 2015**
IRAS project ID: **36467**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper		
Notice of Substantial Amendment (non-CTIMP)	2	11 March 2015
Research protocol or project proposal		4 January 2015

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

NHS
Health Research Authority
NRES Committee East of England - Cambridge South
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Tel: 0115 883 9428

16 February 2015

Dr Ibraheem Hamoodi
New Lister Building Level 2, Room 2. 56
10-16 Alexandra Parade
Glasgow
G31 2er

Dear Dr Hamoodi

Study title:	**A retrospective study to determine if diffusion weighted MRI pre-Uterine Artery Embolisation can predict the change in fibroid volume**
REC reference:	**15/EE/0054**
IRAS project ID:	**164667**

Thank you for your letter of 12 February 2015, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Assistant Nicola Kohut, nrescommittee.eastofengland-cambridgesouth@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

PATIENT INFORMATION SHEET

Uterine Artery Embolisation : Is It an Acceptable Treatment and Why Does it Work?

GROUP 3 - WOMEN HAVING UTERINE ARTERY EMBOLISATION

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives or your general practitioner if you wish.

Ask us if there is anything that is not clear, or, if you would like more information. If you do take part, you will be in one of three different groups that are involved in this study.

1. What is the purpose of the study?

Fibroids are the most common pelvic benign [non-cancerous] tumour of the female genital tract. Heavy periods are one of the most common symptoms of fibroids but how they lead to heavy periods is not clear. Fibroids are usually treated by surgery that often involves hysterectomy [removal of the womb]. Other treatments are now becoming available for those who wish to avoid surgery whilst still maintaining their fertility and one of these is uterine artery embolisation [UAE] which is particularly effective in treating heavy menstrual bleeding [HMB] but how this treatment works is not clear. We are studying groups of women who complain of HMB some who have fibroids and some who do not and will be comparing their results. We will also be studying women before and after UAE which is a fairly new treatment that decreases the menstrual blood loss that is associated with fibroids and we hope to discover how it works.

We would like to invite you to help us to find the cause for the fibroid-associated menstrual problems since this will help researchers to find a proper treatment for this condition.

2. Why have I been chosen?

You have been chosen because you are having uterine artery embolisation as treatment of your fibroids. **You will not be suitable if you are on a hormonal preparation such as the oral contraceptive pill or other hormones taken every day.**

.

3. Do I have to take part?

No, taking part is voluntary. It is up to you to decide whether or not to take part. If you do decide to take part we will ask you to sign a consent form and give you a copy of this information sheet and the consent form to keep. If you decide to take part you are still free

Acute Services Division

Women and Children's Directorate

NHS Greater Glasgow and Clyde

CONSENT FORM

Study Title: **Uterine Artery Embolisation : Is It an Acceptable Treatment and Why Does it Work?**

	Your Consent	(Subject Initials)
1.	I confirm I have read and understand the information sheet dated *March 2010* for the above Study and have had the opportunity to ask questions.	
2.	I understand that my participation is voluntary and that I am free to withdraw without giving any reason, without my medical care or legal rights being affected.	
3.	I understand that the Researchers will need my permission to look at my health records both in respect of the current Study and any further research that may be conducted in relation to it, even if I withdraw. I agree to this access.	
4	I agree that samples of **tissue and blood** taken can be used for future research.	
5	I agree to you notifying my general practitioner of my participation in this study	
6.	I agree to take part in the above study.	

Printed name of patient

Signature of patient
Please date your own signature at the time of signing.

Date .....

Printed name of person explaining consent

Signature of person explaining consent

Date .....

Delivering better health

www.nhsggc.org.uk

Baseline Questionnaire

Study number ☐ Date of birth ☐

Height (cms) ☐ Weight (kg) ☐

1. Marital status

single	☐ 1
married	☐ 2
steady partner	☐ 3
separated	☐ 4
divorced	☐ 5
widowed	☐ 6

2. How long have you had heavy periods for?

Less than six months	☐ 1
six to twelve months	☐ 2
one to two years	☐ 3
two to five years	☐ 4
over five years	☐ 5

3. Are your periods regular? (once a month)

yes	☐ 1
no	☐ 2

4. How long is it from the first day of one period to the first day of the next?

more than six weeks	☐ 1
four to six weeks	☐ 2
three to four weeks	☐ 3
less than three weeks	☐ 4
totally unpredictable	☐ 5

5. How long do your periods last?

less than three days	☐ 1
three to five days	☐ 2
five to seven days	☐ 3
seven to ten days	☐ 4
more than ten days	☐ 5
no bleeding at all	☐ 6

6. For how many days is the period heavy? ☐☐

7. Are your periods painful?

yes	☐ 1
no	☐ 2
sometimes	☐ 3

8. If painful, for how many days do you have pain? ☐☐

9. Do you take pain-killers for period pain?

yes	☐ 1
no	☐ 2

Group 3: Follow up Questionnaire.

1. Following your treatment have your periods

stopped?	1
continued, but lighter?	2
stayed the same?	3
continued, but heavier	4

if your periods have stopped go to question 8

2. How long is it from the first day of one period to the first day of the next?

more than six weeks	1
four to six weeks	2
three to four weeks	3
less than three weeks	4
totally unpredictable	5

3. How long do your periods last?

less than three days	1
three to five days	2
five to seven days	3
seven to ten days	4
more than ten days	5

4. For how many days is the period heavy? ☐☐

5. Do you have pain with your period?

no	1
less than before	2
same as before	3
worse than before	4

6. If painful, for how many days do you have pain? ☐☐

7. Do you take pain-killers for period pain?

yes	1
no	2

8. For each day of your period please show how severe the symptoms were by giving a score of between 1 - 5 (1 = mild bleeding or pain, 5 = the worst bleeding or pain you can think of)

Day of period	bleeding score	pain score
1	☐	☐
2	☐	☐
3	☐	☐
4	☐	☐
5	☐	☐
6	☐	☐
7	☐	☐
8	☐	☐
9	☐	☐
10	☐	☐

Uterine Artery Embolization Study

Menstrual Blood Loss Diary - Please complete for a period prior to UAE

Please would you record your use of tampons and sanitary towels in the table below.

Date period started: ...../...../.....

Brand _____and Type_____ of **towel** you used on days _____

Brand _____and Type_____ of **towel** you used on days _____

Brand _____and Type_____ of **tampon** you used on days _____

Brand _____and Type_____ of **tampon** you used on days _____

(e.g. Tampax - Super Plus days 1-2 then Tampax - Regular days 3-4)

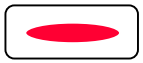
On the next page you will find a Menstrual Blood Loss Diary. Please would you place a tick under the day and next to the box that represents how marked your sanitary materials are each time you change them. Please enter a tick each time you change your tampon or towel.

Record clots by indicating whether they are the size of a **1p** or **50p** coin in the 'clots' row under the relevant day. If you have a clot that is larger than a 50p please record this in comparison to the size of a golf ball (GB).

e.g. under day 1 you may say that you had 1 clot the size of a 50p coin and 3 clots the size of a 1p coin and one clot the size of a golf ball. You would write this as 1 x 50p, 3 x 1p, 1 x GB.

Record any incidences of flooding by placing a tick mark in the flooding row under the relevant day.

PLEASE TURN OVER FOR YOUR DAILY BLOOD LOSS DIARY

TOWEL	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
								
								
								
Clots								
Flooding								
TAMPON	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
								
								
								
Clots								
Flooding								

Appendix 2

Protocol for the Management of Pain and Nausea for patients attending for Uterine Artery Embolisation (UAE)

NHS Greater Glasgow and Clyde

The protocol should be used for all patients undergoing uterine artery embolisation for fibroids. The aim is to minimise pain and nausea through anticipatory prescribing. Prescribing can be undertaken by doctors within the ward setting or doctors within radiology.

Analgesia Plan - Pre-operative checks

Discuss with patient the use of Steroid or Non Steroidal Anti Inflammatory Drugs. (NSAID). Identify any issues that could contraindicate prescribing NSAID therapy including:

- Aspirin sensitivity
- History of Asthma
- Uncontrolled hypertension
- Hypovolaemia
- Acute renal failure
- History of upper gastro intestinal ulceration or intolerance of NSAID's
- Clotting abnormalities/coagulopathy

If the patient describes a history of Gastro Oesophageal Reflux Disease (GORD), consider prescribing Omeprazole 20mg daily as cover for NSAID and Steroid Therapy

Procedure - Pre-operative Prescription

- Oxycontin 10mg orally at least 1 hour before procedure and after formal written informed consent has been obtained
- Diclofenac Modified Release (M.R.) 75mg orally
- Ondansetron 8mg orally
- Paracetamol 1g orally
- Dexamethasone 8mg orally
- Omeprazole 20mg orally if required

Intra-procedural therapy

- Midazolam dosage determined as per protocol if required for anxiety
- Morphine 10mg subcutaneously at commencement of Embolisation of first vessel
- Fentanyl (as per Diagnostic Directorate Protocol)
- Morphine 10mg subcutaneously on removal of vascular sheath, but only if more than 1 hour since last dose

Post Procedure - REGULAR PRESCRIPTIONS

- Oxycontin 10mg orally at 10pm
- Diclofenac M.R. 75mg orally 12 hourly from 10pm
- Paracetamol 1g orally 6 hourly. If patient actively vomiting, consider changing to IV or PR administration. Consultant prescription for IV Paracetamol MUST be obtained. Change back to oral route immediately after the vomiting stops. Refer to therapeutics handbook
- **N.B. IV PARACETAMOL MUST BE DOSE ADJUSTED FOR PATIENTS WEIGHING LESS THAN 50KG – REFER TO THERAPEUTICS HANDBOOK**
- Ondansetron 4mg Oral/IV 8 hourly (should be given regularly to prevent nausea)

Post Procedure - AS REQUIRED PRESCRIPTIONS

- Oxynorm 5mg orally – up to hourly as required
- Cyclizine 50mg Oral/Iv/IM 8 hourly
- Prochloperazine 6mg Buccal 12 hourly

Discharge medication

5 day supply:

- Cocodamol 30/500 tabs 2 tablets 4 to 6 hourly as required
- Diclofenac M.R. 75mg 12 hourly
- Omeprazole 20mg daily if required for duration of NSAID therapy only

Any queries to be directed to the acute pain nurse or duty anaesthetist if out of hours:

North West Duty Anaesthetist – (page 5106)
South Duty Anaesthetist – (page 7307)
North West Acute Pain Nurse – (page 5258)
South Acute Pain Nurse – (page 7157)

This clinical protocol was developed by Dr Grant Urquhart, Consultant Radiologist, Southern General Hospital as Chair of a short life Greater Glasgow & Clyde working group.

This protocol replaces all previous local guidance relating to uterine artery embolisation.

Approved by: Short life working group
Date of Review: November 2013

Created by June Leckie Clinical Effectiveness Facilitator, Victoria Infirmary Tel: 0141 201 5441
Designed by Medical Illustration Services Tel: 0141 211 4692 • MIS 245560

Appendix 3

thebmj

BMJ 2015;351:h4887 doi: 10.1136/bmj.h4887 (Published 13 October 2015)

CLINICAL REVIEW

Fibroids: diagnosis and management

Mary Ann Lumsden *professor of gynaecology and medical education*[1], Ibraheem Hamoodi *specialist trainee and research fellow*[1], Janesh Gupta *professor of obstetrics and gynaecology*[2], Martha Hickey *professor of obstetrics and gynaecology*[3]

[1]University of Glasgow, Glasgow Royal Infirmary Campus, Glasgow G31 2ER, UK; [2]University of Birmingham, Birmingham Women's Hospital, Birmingham, UK; [3]The University of Melbourne and the Royal Women's Hospital, Royal Women's Hospital, Parkville, Melbourne, Australia

Uterine leiomyomas (fibroids) are the most common benign tumours in women. They may be single or multiple and their size varies from a few millimetres to 30 cm or more. By age 50 nearly 70% of white women and more than 80% of black women have had at least one fibroid.[1] Box 1 lists the several risk factors for fibroids. Symptomatic fibroids are often managed surgically, and this confers a considerable burden on healthcare costs.[2] This review aims to update non-specialists on the investigation and management of fibroids. Gaps in current knowledge are highlighted.

What are fibroids and where are they found?

Fibroids are a mixture of smooth muscle cells and fibroblasts, which form hard, round, whorled tumours in the myometrium. The pathophysiology of fibroids remains unknown, although it is hypothesised that each fibroid is derived from a mutation in a single smooth muscle cell.[3]

The uterus is the commonest site for fibroids (fig 1⇓). The location may have an effect on symptoms and quality of life. For example, submucous fibroids may lead to heavy menstrual bleeding and fertility problems and large fibroids may occupy two or more locations and can extend from the endometrial cavity to the serosal surface.

What controls the growth of fibroids?

Oestrogen and progesterone control the proliferation and maintenance of uterine fibroids, and most medical treatments act by inhibiting the production of sex steroids or their action. The primary action of oestrogen is thought to be mediated through induction of progesterone receptor expression, thereby allowing leiomyomas to respond to progesterone.[4] Hormonal replacement therapy may cause some growth of fibroids, but this is of uncertain clinical importance.[10]

What is the clinical course of uterine fibroids?

Fibroids are rare in girls before menarche and regress after the menopause. One retrospective study of 122 premenopausal women who had at least two transvaginal ultrasound scans over a median interval of two years reported that fibroids tended to grow by around 35% of their volume each year, and that small fibroids (<2 cm) or intramural fibroids grew most quickly,[11] although this was variable.

How do women with fibroids present?

Fibroids tend to be asymptomatic. When symptoms do occur, however, menstrual problems, particularly heavy menstrual bleeding and pressure symptoms, are typical (box 2)[12] and can have a negative effect on quality of life. They usually require treatment.[13] The size of fibroids does not necessarily determine symptoms.

When do fibroids need to be investigated?

Fibroids are common, and with the widespread availability of high resolution ultrasonography, they are often diagnosed incidentally.

Women presenting in primary care with symptoms suggestive of fibroids should have their gynaecological history evaluated, including cervical screening, and should undergo a pelvic examination for any masses, a haemoglobin estimation to check for iron deficiency anaemia, and, if urinary symptoms are present, midstream urine testing to exclude a urinary tract infection. Diagnostic uncertainty, association with problematic symptoms, or any clinical or radiological suspicion of malignancy should prompt referral for further investigations. Women with asymptomatic fibroids, if the diagnosis is certain, often do not need further investigation or treatment.

Correspondence to: M A Lumsden Maryann.Lumsden@glasgow.ac.uk

Extra material supplied by the author (see http://www.bmj.com/content/351/bmj.h4887?tab=related#datasupp)

References w1-w34 are available on bmj.com

Is the size of the dominant fibroid & it's location a determining factor in the success of uterine artery embolization (UAE) ?

Dr Ibraheem Hamoodi[1], Prof Mary Ann Lumsden[1], Prof Jon Moss[2]
[1] The University of Glasgow [2] Gartnavel General Hospital, Glasgow

Introduction:

Uterine artery embolisation (UAE) is a non-invasive treatment for uterine fibroids. *Ravina et al* [1] was first to report that embolisation of the uterine arteries in a fibroid uterus would lead to avascular necrosis of the fibroid, causing volume reduction in the fibroid uterus and fibroids . Magnetic Resonance Imaging (MRI) is considered the most accurate imaging technique to assess symptomatic uterine fibroids and to evaluate the efficacy of UAE [2].

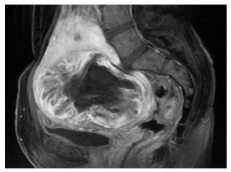


Fig1. Fibroid showing contrast enhancement pre-UAE

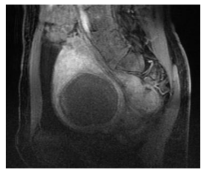


Fig 2. Fibroid showing no contrast enhancement post-UAE

Aim:

To establish if the response of a fibroid uterus to UAE is subject to factors such as the size and location of the dominant fibroid pre-UAE. This will help in stratifying patients prior to having the UAE procedure and avoiding this treatment modality in those that may not have a good response.

Methods:

A five year retrospective study (2010 to 2014) of woman who underwent UAE as a treatment for uterine fibroids that fit the study criteria (n=134). The study looked at the pre and post uterine size, fibroid size and location of fibroids and the complications at 6 months post UAE. The location and the size of the fibroids in each patient were ascertained using T2-weighted contrast enhanced MRI in both sagittal and coronal planes. The fibroid volume and the uterine volume were calculated using the standard prolate ellipse formula. Dominant fibroid shrinkage and fibroid-uterus shrinkage was measured by subtracting 6 months post-UAE volumes from pre-UAE volumes. We tried to identify any correlation between the size and location of the dominant fibroid and its response post UAE.

Results

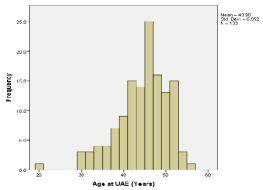


Fig 3. Age at UAE

Location of dominant fibroid	N	Shrinkage Mean+/-STD	Median
Intramural	70	-42.7 +/- 24.5	-41.49
Subserosal	42	-37.7 +/- 27.7	-37.78
Submucosal	9	-69.9 +/- 20.5	-71.83
Intramural with Submucosal element	6	-62.3 +/- 37.0	-59.4

Fig 4. Dominant fibroid shrinkage according to location

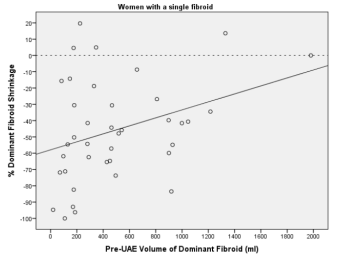


Fig 5. Dominant fibroid shrinkage for women presenting with a single fibroid

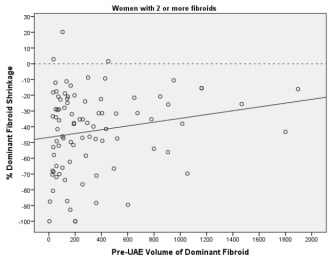


Fig 6. Dominant fibroid shrinkage for women presenting with multiple fibroids

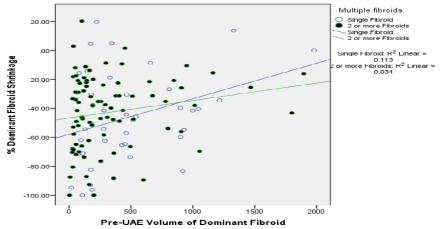


Fig 7. The difference in dominant fibroid shrinkage based on presenting number of fibroids

Single fibroid analysis

ANOVA

Model		Sum of Squares	df	Mean Square	F	P
1	Regression	1938.672	1	1938.672	3.004	0.087
	Residual	55503.347	86	645.388		
	Total	57442.019	87			

Mulitple fibroid analysis

ANOVA

Model		Sum of Squares	df	Mean Square	F	P
1	Regression	4178.716	1	4178.716	4.725	0.036
	Residual	32720.978	37	884.351		
	Total	36899.694	38			

Conclusion :

We found that fibroids closer to the endometrial cavity (submucosal fibroids) tend to shrink more than fibroids further away (subserosal fibroid). This was an observational result as to reach statistical significance more numbers were needed. The peak age of undergoing UAE in this study was 45 years. This relatively late presentation to undergoing UAE may be due to some women seeking different treatment options prior to UAE such as medical treatments. The percentage of both uterine volume shrinkage and dominant fibroid shrinkage seem to suggest that although most women seek relief from HMB, it's the pressure symptoms from a space occupying mass that may be helped by fibroid shrinkage. It maybe that UAE should also target women with fibroids that don't have HMB as a main symptom and offer them an alternative to surgery. Our study suggests that women who present with a single fibroid uterus have a higher rate of shrinkage than those with a multi-fibroid uterus. We also found no correlation between the age of a woman having UAE and the shrinkage of the fibroids.

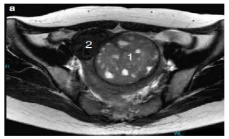
References:
1 Ravina J H, Herbreteau D, Ciraru-Vigneron N, et al. Arterial embolisation to treat uterine myomata. Lancet. 1995;346:671–672
2 Dueholm et al. Reproducibility of evaluation of the uterus by TVS, hysterosonographic examination, hysteroscopy & magnetic resonance imaging. Humreprod. 2002;17:195-200

Can Diffusion Weighted Magnetic Resonance Imaging (DW MRI) help in predicting the outcome of uterine artery embolisation (UAE)?

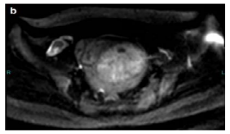
Dr Ibraheem Hamoodi[1], Prof Mary Ann Lumsden[1], Prof Jon Moss[2] , Dr Gillian Macnaught[3]
[1] The University of Glasgow [2] Gartnavel General Hospital, Glasgow [3] The University of Edinburgh

Introduction:

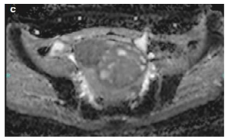
Diffusion-weighted imaging (DWI) is a non-contrast enhanced functional MRI technique currently available in the majority of clinical MRI units. It can provide information on the motion of water molecules in tissues. It permits the quantitative evaluation of tumour tissue such as fibroids. This is through a quantitative parameter, called apparent diffusion coefficient (ADC). The ADC maps that are generated using DWI provide accurate quantification of the cellular motion of water molecules[1]. This movement is dependent on the cellularity of the tissue/lesion allowing tissue characterisation, and possibly reflecting cell death [2]. DWI is a well-established technique in cerebral imaging [3]. However, its application to imaging of other parts of the body is new. It is now being used as a part of the imaging protocols for pelvic malignancies and fibroids [4.]



Fibroid uterus on MRI



Fibroid uterus on DWI MRI



DWI MRI after applying ADC map

Aim:

The aim of this study was to assess whether using DWI offered any additional information over a standard MRI pelvis when imaging uterine fibroids and if the ADC value can be used as a predictor of the response of the fibroid uterus to UAE.

Methods:

Data was extracted from DW MRI images of patients (n=20) relating to a pilot study that compared two embolic agents (Gelfoam and Embosphere) used in UAE. Image analysis was carried out on a GE workstation (ADW 4.0; GE Healthcare) & only the dominant fibroid was evaluated in each patient. The following variables were assessed pre-UAE, 24 hours post UAE and 6 months post-UAE: uterine volume, dominant fibroid volume, endometrial thickness, ADC measurement of dominant fibroid, myometrium and when possible for the endometrium. To calculate the ADC value, a circular region of interest (ROI) was drawn encompassing the region of interest (ROI).

Results:

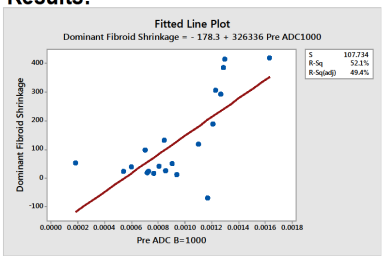


Fig 1: Correlation between Pre-UAE ADC & dominant fibroid volume reduction

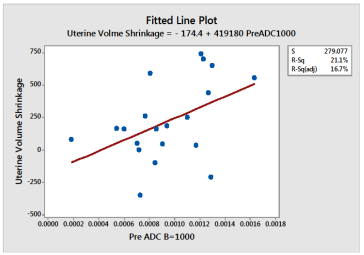


Fig 2: Correlation between Pre-UAE ADC & Uterine volume shrinkage

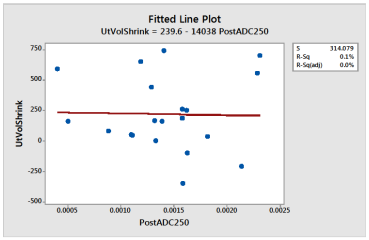


Fig 3: Correlation between uterine volume reduction and post UAE ADC values

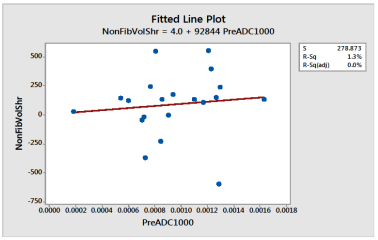


Fig 4: Correlation between Non Fibroid Uterine Volume Shrinkage and Pre UAE ADC

There was a significant reduction of dominant fibroid volume (mm^3) post-UAE procedure (441+/-110 to 314+/-90.8, p= 0.001). A significant correlation between dominant fibroid volume shrinkage (39.15+/-25.72) and pre/post UAE ADC values was found (R-sq=52.1). The relationship is much stronger between dominant fibroid volume shrinkage and pre-UAE (pre-UAE ADC p value < 0.001 while post UAE p-value =0.04).
As for Fibroid uterus volume reduction, there was a significant reduction in the fibroid uterus volume (mm^3) post-UAE procedure (908+/-635 to 688+/-552), p= 0.005). However, pre-UAE ADC seems to explain much more of the variability in dominant fibroid volume shrinkage (R-sq=52.1) than it does for uterine volume shrinkage (R-sq=21.1).
There was also no significant difference in endometrial thickness pre and post UAE (4.36+/-1.9, 4.46+/-2.2, p=0.8) nor was there a difference in the ADC values in the patients that we were able to measure. We also found was no significant difference in ADC values of the myometrium pre-UAE and 6 months post UAE (p=0.096).

Conclusion :

We attempted to measure the change that UAE causes to the fibroid uterus.
The pre-UAE ADC values of the dominant fibroids showed correlation with fibroid shrinkage. However, we were unable with this limited sample to develop an algorithm that predicts uterine fibroid shrinkage. This may be possible with a larger sample however variables such as the level and site of necrosis a fibroid has already undergone may need to be taken into consideration.
The ADC values of the endometrium and the myometrium were not affected indicating that cellular integrity in these layers was unaffected at 6 months post-UAE. This may help reassure women undertaking UAE whom maintaining fertility is a deciding factor. This will need to be assessed again in a larger study to form any meaningful conclusions on this matter.

References:

1 Le Bihan, D., et al., *Diffusion MR imaging: clinical applications.* AJR Am J Roentgenol, 1992. **159**(3): p. 591-9.
2 Mannelli, L., et al., *Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver.* AJR Am J Roentgenol, 2009. **193**(4): p. 1044-52.
3 Muir, K.W., et al., *Imaging of acute stroke.* Lancet Neurol, 2006. **5**(9): p. 755-68.
4 Whittaker, C.S., et al., *Diffusion-weighted MR imaging of female pelvic tumors: a pictorial review.* Radiographics, 2009. **29**(3): p. 759-74; discussion 774-8.

List of References

1. Press, M.F., et al., *Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle.* Lab Invest, 1984. **51**(5): p. 495-503.
2. Kostiuchek, I.N., et al., *[Expression of estrogen and progesterone receptors in the endometrium of fertile and infertile women].* Arkh Patol, 2011. **73**(5): p. 30-2.
3. Bergeron, C., et al., *Immunocytochemical study of progesterone receptors in the human endometrium during the menstrual cycle.* Lab Invest, 1988. **59**(6): p. 862-9.
4. Kearns, M. and P.K. Lala, *Life history of decidual cells: a review.* Am J Reprod Immunol, 1983. **3**(2): p. 78-82.
5. Ferenczy, A. and C. Bergeron, *Histology of the human endometrium: from birth to senescence.* Ann N Y Acad Sci, 1991. **622**: p. 6-27.
6. Christiaens, G.C., *J.E. Markee: menstruation in intraocular endometrial transplants in the rhesus monkey.* Eur J Obstet Gynecol Reprod Biol, 1982. **14**(1): p. 63-5.
7. Rogers, P.A. and C.E. Gargett, *Endometrial angiogenesis.* Angiogenesis, 1998. **2**(4): p. 287-94.
8. Perrot-Applanat, M., et al., *Ovarian steroids in endometrial angiogenesis.* Steroids, 2000. **65**(10-11): p. 599-603.
9. Iruela-Arispe, M.L., J.C. Rodriguez-Manzaneque, and G. Abu-Jawdeh, *Endometrial endothelial cells express estrogen and progesterone receptors and exhibit a tissue specific response to angiogenic growth factors.* Microcirculation, 1999. **6**(2): p. 127-40.
10. Critchley, H.O., et al., *Estrogen receptor beta, but not estrogen receptor alpha, is present in the vascular endothelium of the human and nonhuman primate endometrium.* J Clin Endocrinol Metab, 2001. **86**(3): p. 1370-8.
11. Kelly, F.D., S.A. Tawia, and P.A. Rogers, *Immunohistochemical characterization of human endometrial microvascular basement membrane components during the normal menstrual cycle.* Hum Reprod, 1995. **10**(2): p. 268-76.
12. Cao, W., et al., *Progesterone withdrawal up-regulates fibronectin and integrins during menstruation and repair in the rhesus macaque endometrium.* Hum Reprod, 2007. **22**(12): p. 3223-31.
13. Henriet, P., H.P. Gaide Chevronnay, and E. Marbaix, *The endocrine and paracrine control of menstruation.* Mol Cell Endocrinol, 2012. **358**(2): p. 197-207.
14. Boonyaratanakornkit, V. and D.P. Edwards, *Receptor mechanisms mediating non-genomic actions of sex steroids.* Semin Reprod Med, 2007. **25**(3): p. 139-53.
15. Detmar, M., et al., *Hypoxia regulates the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptors in human skin.* J Invest Dermatol, 1997. **108**(3): p. 263-8.
16. Neufeld, G., et al., *Vascular endothelial growth factor (VEGF) and its receptors.* Faseb j, 1999. **13**(1): p. 9-22.
17. Smith, W.L., D.L. DeWitt, and R.M. Garavito, *Cyclooxygenases: structural, cellular, and molecular biology.* Annu Rev Biochem, 2000. **69**: p. 145-82.
18. Smyth, E.M., et al., *Prostanoids in health and disease.* J Lipid Res, 2009. **50 Suppl**: p. S423-8.
19. Narumiya, S., Y. Sugimoto, and F. Ushikubi, *Prostanoid receptors: structures, properties, and functions.* Physiol Rev, 1999. **79**(4): p. 1193-226.
20. Forman, B.M., et al., *15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma.* Cell, 1995. **83**(5): p. 803-12.
21. Rossi, A., et al., *Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase.* Nature, 2000. **403**(6765): p. 103-8.

22. Bishop-Bailey, D. and T. Hla, *Endothelial cell apoptosis induced by the peroxisome proliferator-activated receptor (PPAR) ligand 15-deoxy-Delta12, 14-prostaglandin J2.* J Biol Chem, 1999. **274**(24): p. 17042-8.
23. Jones, R.L., R.W. Kelly, and H.O. Critchley, *Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation.* Hum Reprod, 1997. **12**(6): p. 1300-6.
24. Critchley, H.O., et al., *Role of inflammatory mediators in human endometrium during progesterone withdrawal and early pregnancy.* J Clin Endocrinol Metab, 1999. **84**(1): p. 240-8.
25. Smith, O.P., H.N. Jabbour, and H.O. Critchley, *Cyclooxygenase enzyme expression and E series prostaglandin receptor signalling are enhanced in heavy menstruation.* Hum Reprod, 2007. **22**(5): p. 1450-6.
26. Gargett, C.E., et al., *Lack of correlation between vascular endothelial growth factor production and endothelial cell proliferation in the human endometrium.* Hum Reprod, 1999. **14**(8): p. 2080-8.
27. Gordon, J.D., et al., *Angiogenesis in the human female reproductive tract.* Obstet Gynecol Surv, 1995. **50**(9): p. 688-97.
28. Uluer, E.T., et al., *The role of hypoxia related angiogenesis in uterine smooth muscle tumors.* Biotech Histochem, 2015. **90**(2): p. 102-10.
29. Triggle, C.R., et al., *The endothelium: influencing vascular smooth muscle in many ways.* Can J Physiol Pharmacol, 2012. **90**(6): p. 713-38.
30. Campbell, J.H. and G.R. Campbell, *Endothelial cell influences on vascular smooth muscle phenotype.* Annu Rev Physiol, 1986. **48**: p. 295-306.
31. Jabbour, H.N., et al., *Inflammatory pathways in female reproductive health and disease.* Reproduction, 2009. **138**(6): p. 903-19.
32. Key, T.J. and M.C. Pike, *The dose-effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk.* Br J Cancer, 1988. **57**(2): p. 205-12.
33. Markee, J.E., *Menstruation in intraocular endometrial transplants in the Rhesus monkey.* Am J Obstet Gynecol, 1978. **131**(5): p. 558-9.
34. Jabbour, H.N., et al., *Endocrine regulation of menstruation.* Endocr Rev, 2006. **27**(1): p. 17-46.
35. Lumsden, M.A., et al., *The concentrations of prostaglandins in endometrium during the menstrual cycle in women with measured menstrual blood loss.* Prostaglandins Leukot Med, 1986. **23**(2-3): p. 217-27.
36. Szostek, A.Z., et al., *Interleukins affect equine endometrial cell function: modulatory action of ovarian steroids.* Mediators Inflamm, 2014. **2014**: p. 208103.
37. NICE, *Heavy Menstrual Bleeding.* March 2012. **Clinical Guidline 44**.
38. Santer, M., P. Warner, and S. Wyke, *A Scottish postal survey suggested that the prevailing clinical preoccupation with heavy periods does not reflect the epidemiology of reported symptoms and problems.* J Clin Epidemiol, 2005. **58**(11): p. 1206-10.
39. Bhattacharya, S., et al., *Hysterectomy, endometrial ablation and Mirena(R) for heavy menstrual bleeding: a systematic review of clinical effectiveness and cost-effectiveness analysis.* Health Technol Assess, 2011. **15**(19): p. iii-xvi, 1-252.
40. Hallberg, L., et al., *Menstrual blood loss and iron deficiency.* Acta Med Scand, 1966. **180**(5): p. 639-50.
41. Fraser, I.S., et al., *Efficacy of mefenamic acid in patients with a complaint of menorrhagia.* Obstet Gynecol, 1981. **58**(5): p. 543-51.
42. RCOG, *National Heavy Menstrual Bleeding Auidt.* 2012.
43. Fraser, I.S., et al., *Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding?* Hum Reprod, 2007. **22**(3): p. 635-43.

44. Diaz, R., et al., *Hemostatic abnormalities in young females with heavy menstrual bleeding.* J Pediatr Adolesc Gynecol, 2014. **27**(6): p. 324-9.
45. Schatz, F., et al., *Implications of decidualization-associated protease expression in implantation and menstruation.* Semin Reprod Endocrinol, 1999. **17**(1): p. 3-12.
46. Wu, Q. and Z. Zhao, *Inhibition of PAI-1: a new anti-thrombotic approach.* Curr Drug Targets Cardiovasc Haematol Disord, 2002. **2**(1): p. 27-42.
47. Rybo, G. and L. Hallberg, *Influence of heredity and environment on normal menstrual blood loss. A study of twins.* Acta Obstet Gynecol Scand, 1966. **45**(4): p. 389-410.
48. Clancy, R., et al., *Nitric oxide synthase/COX cross-talk: nitric oxide activates COX-1 but inhibits COX-2-derived prostaglandin production.* J Immunol, 2000. **165**(3): p. 1582-7.
49. Chiarugi, V., L. Magnelli, and O. Gallo, *Cox-2, iNOS and p53 as play-makers of tumor angiogenesis (review).* Int J Mol Med, 1998. **2**(6): p. 715-9.
50. Fraser, I.S., *Prostaglandins, prostaglandin inhibitors and their roles in gynaecological disorders.* Baillieres Clin Obstet Gynaecol, 1992. **6**(4): p. 829-57.
51. Cameron, I.T., et al., *The effects of mefenamic acid and norethisterone on measured menstrual blood loss.* Obstet Gynecol, 1990. **76**(1): p. 85-8.
52. Kovachev, S.M., S.D. Nikolov, and A.P. Mihova, *Uterine leiomyoma in a man with persistent Mullerian duct syndrome and seminoma.* Isr Med Assoc J, 2014. **16**(11): p. 735-7.
53. Bozini, N. and E.C. Baracat, *The history of myomectomy at the Medical School of University of Sao Paulo.* Clinics (Sao Paulo), 2007. **62**(3): p. 209-10.
54. Siskin, G., *Thieme Medical Publishers Inc.*
Interventional Radiology in Women's Health. 2009: p. 27.
55. Buttram, V.C., Jr. and R.C. Reiter, *Uterine leiomyomata: etiology, symptomatology, and management.* Fertil Steril, 1981. **36**(4): p. 433-45.
56. Baird, D.D., et al., *High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence.* American Journal of Obstetrics and Gynecology, 2003. **188**(1): p. 100-7.
57. Wise, L.A., et al., *Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African-American women: a prospective study.* Am J Epidemiol, 2004. **159**(2): p. 113-23.
58. Cardozo, E.R., et al., *The estimated annual cost of uterine leiomyomata in the United States.* Am J Obstet Gynecol, 2012. **206**(3): p. 211.e1-9.
59. 2012-2013, P., *https://www.gov.uk/government/publications/payment-by-results-pbr-operational-guidance-and-tariffs.* UK Government NHS tariff.
60. Ligon, A.H. and C.C. Morton, *Genetics of uterine leiomyomata.* Genes Chromosomes Cancer, 2000. **28**(3): p. 235-45.
61. Moravek, M.B., et al., *Ovarian steroids, stem cells and uterine leiomyoma: therapeutic implications.* Hum Reprod Update, 2014.
62. Vikhlyaeva, E.M., Z.S. Khodzhaeva, and N.D. Fantschenko, *Familial predisposition to uterine leiomyomas.* Int J Gynaecol Obstet, 1995. **51**(2): p. 127-31.
63. Ross, R.K., et al., *Risk factors for uterine fibroids: reduced risk associated with oral contraceptives.* Br Med J (Clin Res Ed), 1986. **293**(6543): p. 359-62.
64. Evans, P. and S. Brunsell, *Uterine fibroid tumors: diagnosis and treatment.* Am Fam Physician, 2007. **75**(10): p. 1503-8.
65. D'Aloisio, A.A., et al., *Association of intrauterine and early-life exposures with diagnosis of uterine leiomyomata by 35 years of age in the Sister Study.* Environ Health Perspect, 2010. **118**(3): p. 375-81.
66. Chen, C.R., et al., *Risk factors for uterine fibroids among women undergoing tubal sterilization.* Am J Epidemiol, 2001. **153**(1): p. 20-6.
67. Templeman, C., et al., *Risk factors for surgically removed fibroids in a large cohort of teachers.* Fertil Steril, 2009. **92**(4): p. 1436-46.

68. Terry, K.L., et al., *Anthropometric characteristics and risk of uterine leiomyoma.* Epidemiology, 2007. **18**(6): p. 758-63.
69. Gupta, S., J. Jose, and I. Manyonda, *Clinical presentation of fibroids.* Best Pract Res Clin Obstet Gynaecol, 2008. **22**(4): p. 615-26.
70. *Myomas and reproductive function.* Fertil Steril, 2008. **90**(5 Suppl): p. S125-30.
71. Van Voorhis, B., *A 41-year-old woman with menorrhagia, anemia, and fibroids: review of treatment of uterine fibroids.* Jama, 2009. **301**(1): p. 82-93.
72. Puri, K., et al., *Submucosal fibroids and the relation to heavy menstrual bleeding and anemia.* Am J Obstet Gynecol, 2014. **210**(1): p. 38.e1-7.
73. Sulaiman, S., et al., *Uterine fibroids--do size and location determine menstrual blood loss?* Eur J Obstet Gynecol Reprod Biol, 2004. **115**(1): p. 85-9.
74. Sinclair, D.C., A. Mastroyannis, and H.S. Taylor, *Leiomyoma simultaneously impair endometrial BMP-2-mediated decidualization and anticoagulant expression through secretion of TGF-beta3.* J Clin Endocrinol Metab, 2011. **96**(2): p. 412-21.
75. Miller, N.F. and P.P. Ludovici, *On the origin and development of uterine fibroids.* Am J Obstet Gynecol, 1955. **70**(4): p. 720-40.
76. Farrer-Brown, G., J.O. Beilby, and M.H. Tarbit, *Venous changes in the endometrium of myomatous uteri.* Obstet Gynecol, 1971. **38**(5): p. 743-51.
77. Chimbira, T.H., A.B. Anderson, and A. Turnbull, *Relation between measured menstrual blood loss and patient's subjective assessment of loss, duration of bleeding, number of sanitary towels used, uterine weight and endometrial surface area.* Br J Obstet Gynaecol, 1980. **87**(7): p. 603-9.
78. Palmer, S.S., et al., *Increased expression of stromelysin 3 mRNA in leiomyomas (uterine fibroids) compared with myometrium.* J Soc Gynecol Investig, 1998. **5**(4): p. 203-9.
79. Stewart, E.A. and R.A. Nowak, *Leiomyoma-related bleeding: a classic hypothesis updated for the molecular era.* Hum Reprod Update, 1996. **2**(4): p. 295-306.
80. Lai, T.H., et al., *Expression Patterns of VEGF and Flk-1 in Human Endometrium during the Menstrual Cycle.* J Reprod Infertil, 2015. **16**(1): p. 3-9.
81. Farquhar, C., *Do uterine fibroids cause infertility and should they be removed to increase fertility?* Bmj, 2009. **338**: p. b126.
82. Templeton A, C.I., O'Brien PMS, *Evidence -based Fertility Treatment.* London RCOG Press, 1998.
83. Pritts, E.A., W.H. Parker, and D.L. Olive, *Fibroids and infertility: an updated systematic review of the evidence.* Fertility and Sterility, 2009. **91**(4): p. 1215-23.
84. Muram, D., M. Gillieson, and J.H. Walters, *Myomas of the uterus in pregnancy: ultrasonographic follow-up.* Am J Obstet Gynecol, 1980. **138**(1): p. 16-9.
85. Barlow, D.H., et al., *Individualized vaginal bleeding experience of women with uterine fibroids in the PEARL I randomized controlled trial comparing the effects of ulipristal acetate or placebo.* Hum Reprod, 2014. **29**(3): p. 480-9.
86. Fedele, L., et al., *Transvaginal ultrasonography versus hysteroscopy in the diagnosis of uterine submucous myomas.* Obstet Gynecol, 1991. **77**(5): p. 745-8.
87. Vercellini, P., et al., *The role of transvaginal ultrasonography and outpatient diagnostic hysteroscopy in the evaluation of patients with menorrhagia.* Hum Reprod, 1997. **12**(8): p. 1768-71.
88. Wilde, S. and S. Scott-Barrett, *Radiological appearances of uterine fibroids.* Indian J Radiol Imaging, 2009. **19**(3): p. 222-31.
89. Harlow, B.L., N.S. Weiss, and S. Lofton, *The Epidemiology of Sarcomas of the Uterus.* Journal of the National Cancer Institute, 1986. **76**(3): p. 399-402.
90. Quade, B.J., et al., *Molecular pathogenesis of uterine smooth muscle tumors from transcriptional profiling.* Genes Chromosomes Cancer, 2004. **40**(2): p. 97-108.
91. Leibsohn, S., et al., *Leiomyosarcoma in a series of hysterectomies performed for presumed uterine leiomyomas.* American Journal of Obstetrics and Gynecology, 1990. **162**(4): p. 968-74; discussion 974-6.

92. Pritts, E.A., et al., *The prevalence of occult leiomyosarcoma at surgery for presumed uterine fibroids: a meta-analysis.* Gynecol Surg, 2015. **12**(3): p. 165-177.
93. Parker, W.H., *Etiology, symptomatology, and diagnosis of uterine myomas.* Fertil Steril, 2007. **87**(4): p. 725-36.
94. Goto, A., et al., *Usefulness of Gd-DTPA contrast-enhanced dynamic MRI and serum determination of LDH and its isozymes in the differential diagnosis of leiomyosarcoma from degenerated leiomyoma of the uterus.* Int J Gynecol Cancer, 2002. **12**(4): p. 354-61.
95. Sato, K., et al., *Clinical application of diffusion-weighted imaging for preoperative differentiation between uterine leiomyoma and leiomyosarcoma.* American Journal of Obstetrics and Gynecology, 2014. **210**(4): p. 368 e1-8.
96. Sutton, C., *Hysterectomy: a historical perspective.* Baillieres Clin Obstet Gynaecol, 1997. **11**(1): p. 1-22.
97. Philipp, E.E., *Victor Bonney: The Gynaecological Surgeon of the Twentieth Century.* Journal of the Royal Society of Medicine, 2001. **94**(6): p. 311-312.
98. Khan, A.T., M. Shehmar, and J.K. Gupta, *Uterine fibroids: current perspectives.* Int J Womens Health, 2014. **6**: p. 95-114.
99. Eder, S., et al., *Efficacy and safety of oral tranexamic acid in women with heavy menstrual bleeding and fibroids.* Womens Health (Lond Engl), 2013. **9**(4): p. 397-403.
100. Lukes, A.S., et al., *Tranexamic acid treatment for heavy menstrual bleeding: a randomized controlled trial.* Obstet Gynecol, 2010. **116**(4): p. 865-75.
101. Peitsidis, P. and A. Koukoulomati, *Tranexamic acid for the management of uterine fibroid tumors: A systematic review of the current evidence.* World J Clin Cases, 2014. **2**(12): p. 893-8.
102. Caglar, G.S., et al., *Intravenous tranexamic acid use in myomectomy: a prospective randomized double-blind placebo controlled study.* Eur J Obstet Gynecol Reprod Biol, 2008. **137**(2): p. 227-31.
103. Sentilhes, L., et al., *Tranexamic acid for the prevention and treatment of postpartum haemorrhage.* Br J Anaesth, 2015. **114**(4): p. 576-87.
104. Lethaby, A., K. Duckitt, and C. Farquhar, *Non-steroidal anti-inflammatory drugs for heavy menstrual bleeding.* Cochrane Database Syst Rev, 2013. **1**: p. Cd000400.
105. Edgren, R.A. and F.Z. Stanczyk, *Nomenclature of the gonane progestins.* Contraception, 1999. **60**(6): p. 313.
106. Nilsson, C.G., E.D. Johansson, and T. Luukkainen, *A D-norgestrel-releasing IUD.* Contraception, 1976. **13**(4): p. 503-14.
107. Youm, J., et al., *Factors affecting the spontaneous expulsion of the levonorgestrel-releasing intrauterine system.* Int J Gynaecol Obstet, 2014. **126**(2): p. 165-9.
108. Kriplani, A., et al., *Efficacy of the levonorgestrel-releasing intrauterine system in uterine leiomyoma.* Int J Gynaecol Obstet, 2012. **116**(1): p. 35-8.
109. Magalhaes, J., J.M. Aldrighi, and G.R. de Lima, *Uterine volume and menstrual patterns in users of the levonorgestrel-releasing intrauterine system with idiopathic menorrhagia or menorrhagia due to leiomyomas.* Contraception, 2007. **75**(3): p. 193-8.
110. Marshall, J.C. and M.L. Griffin, *The role of changing pulse frequency in the regulation of ovulation.* Hum Reprod, 1993. **8 Suppl 2**: p. 57-61.
111. Jayes, F.C., J.H. Britt, and K.L. Esbenshade, *Role of gonadotropin-releasing hormone pulse frequency in differential regulation of gonadotropins in the gilt.* Biol Reprod, 1997. **56**(4): p. 1012-9.
112. Magon, N., *Gonadotropin releasing hormone agonists: Expanding vistas.* Indian J Endocrinol Metab, 2011. **15**(4): p. 261-7.
113. Bedaiwy, M.A., N.A. Mousa, and R.F. Casper, *Aromatase inhibitors prevent the estrogen rise associated with the flare effect of gonadotropins in patients treated with GnRH agonists.* Fertil Steril, 2009. **91**(4 Suppl): p. 1574-7.
114. *Agents stimulating gonadal function in the human. Report of a WHO scientific group.* World Health Organ Tech Rep Ser, 1973. **514**: p. 1-30.

115. Moroni, R., et al., *Pharmacological treatment of uterine fibroids.* Ann Med Health Sci Res, 2014. **4**(Suppl 3): p. S185-92.
116. De Falco, M., et al., *[GnRH agonists and antagonists in the preoperative therapy of uterine fibroids: literature review].* Minerva Ginecol, 2006. **58**(6): p. 553-60.
117. Lethaby, A., B. Vollenhoven, and M. Sowter, *Pre-operative GnRH analogue therapy before hysterectomy or myomectomy for uterine fibroids.* Cochrane Database Syst Rev, 2000(2): p. CD000547.
118. Sinai Talaulikar, V., A.M. Belli, and I. Manyonda, *GnRH agonists: do they have a place in the modern management of fibroid disease?* J Obstet Gynaecol India, 2012. **62**(5): p. 506-10.
119. Mavrelos, D., et al., *The value of pre-operative treatment with GnRH analogues in women with submucous fibroids: a double-blind, placebo-controlled randomized trial.* Hum Reprod, 2010. **25**(9): p. 2264-9.
120. Freundl, G., et al., *Steroidal 'add-back' therapy in patients treated with GnRH agonists.* Gynecol Obstet Invest, 1998. **45 Suppl 1**: p. 22-30; discussion 35.
121. Whitaker, L.H., A.R. Williams, and H.O. Critchley, *Selective progesterone receptor modulators.* Curr Opin Obstet Gynecol, 2014. **26**(4): p. 237-42.
122. Carbonell, J.L., et al., *Treatment of Uterine Myoma with 2.5 or 5 mg Mifepristone Daily during 3 Months with 9 Months Posttreatment Followup: Randomized Clinical Trial.* ISRN Obstet Gynecol, 2013. **2013**: p. 649030.
123. Talaulikar, V.S. and I.T. Manyonda, *Ulipristal acetate: a novel option for the medical management of symptomatic uterine fibroids.* Adv Ther, 2012. **29**(8): p. 655-63.
124. Donnez, J., et al., *Ulipristal acetate versus placebo for fibroid treatment before surgery.* N Engl J Med, 2012. **366**(5): p. 409-20.
125. Donnez, J., et al., *Long-term treatment of uterine fibroids with ulipristal acetate.* Fertil Steril, 2014. **101**(6): p. 1565-73.e1-18.
126. Donnez, J., et al., *Efficacy and safety of repeated use of ulipristal acetate in uterine fibroids.* Fertil Steril, 2015. **103**(2): p. 519-27.e3.
127. Spitz, I.M., *Clinical utility of progesterone receptor modulators and their effect on the endometrium.* Curr Opin Obstet Gynecol, 2009. **21**(4): p. 318-24.
128. Williams, A.R., et al., *Endometrial morphology after treatment of uterine fibroids with the selective progesterone receptor modulator, ulipristal acetate.* Int J Gynecol Pathol, 2012. **31**(6): p. 556-69.
129. Brazert, M., M.P. Korman, and L.A. Pawelczyk, *[Applicability of selective progesterone receptor modulators in the treatment of uterine leiomyomata and their future role in the field of gynecology].* Ginekol Pol, 2013. **84**(9): p. 794-800.
130. Luyckx, M., et al., *First series of 18 pregnancies after ulipristal acetate treatment for uterine fibroids.* Fertil Steril, 2014. **102**(5): p. 1404-9.
131. Varelas, F.K., et al., *The effect of anastrazole on symptomatic uterine leiomyomata.* Obstet Gynecol, 2007. **110**(3): p. 643-9.
132. Ravina, J.H., et al., *Arterial embolisation to treat uterine myomata.* Lancet, 1995. **346**(8976): p. 671-2.
133. Gupta, J.K., et al., *Uterine artery embolization for symptomatic uterine fibroids.* Cochrane Database Systematic Review, 2012. **5**: p. Cd005073.
134. Manyonda, I.T., et al., *Uterine artery embolization versus myomectomy: impact on quality of life--results of the FUME (Fibroids of the Uterus: Myomectomy versus Embolization) Trial.* Cardiovasc Intervent Radiol, 2012. **35**(3): p. 530-6.
135. Kaump, G.R. and J.B. Spies, *The impact of uterine artery embolization on ovarian function.* J Vasc Interv Radiol, 2013. **24**(4): p. 459-67.
136. Stewart, E.A., et al., *Focused ultrasound treatment of uterine fibroid tumors: safety and feasibility of a noninvasive thermoablative technique.* Am J Obstet Gynecol, 2003. **189**(1): p. 48-54.

137. Hynynen, K., et al., *A clinical, noninvasive, MR imaging-monitored ultrasound surgery method.* Radiographics, 1996. **16**(1): p. 185-95.
138. Chung, A.H., F.A. Jolesz, and K. Hynynen, *Thermal dosimetry of a focused ultrasound beam in vivo by magnetic resonance imaging.* Med Phys, 1999. **26**(9): p. 2017-26.
139. Fan, R., et al., *[Prospective study on magnetic resonance-guided focused ultrasound surgery for symptomatic uterine fibroid: short-term follow up].* Zhonghua Fu Chan Ke Za Zhi, 2013. **48**(3): p. 183-7.
140. Voogt, M.J., et al., *Volumetric feedback ablation of uterine fibroids using magnetic resonance-guided high intensity focused ultrasound therapy.* Eur Radiol, 2012. **22**(2): p. 411-7.
141. Froeling, V., et al., *Midterm results after uterine artery embolization versus MR-guided high-intensity focused ultrasound treatment for symptomatic uterine fibroids.* Cardiovasc Intervent Radiol, 2013. **36**(6): p. 1508-13.
142. Gizzo, S., et al., *Magnetic resonance-guided focused ultrasound myomectomy: safety, efficacy, subsequent fertility and quality-of-life improvements, a systematic review.* Reprod Sci, 2014. **21**(4): p. 465-76.
143. Garza Leal, J.G., et al., *Laparoscopic ultrasound-guided radiofrequency volumetric thermal ablation of symptomatic uterine leiomyomas: feasibility study using the Halt 2000 Ablation System.* J Minim Invasive Gynecol, 2011. **18**(3): p. 364-71.
144. Berman, J.M., et al., *Three-year outcome of the Halt trial: a prospective analysis of radiofrequency volumetric thermal ablation of myomas.* J Minim Invasive Gynecol, 2014. **21**(5): p. 767-74.
145. Salama, S.S. and G.S. Kilic, *Uterine fibroids and current clinical challenges.* J Turk Ger Gynecol Assoc, 2013. **14**(1): p. 40-5.
146. Hysterectomy-association.org.uk, *http://www.hysterectomy-association.org.uk/research/latest-hysterectomy-statistics-in-uk-for-the-year-2011-to-2012/.* 2013.
147. Okolo, S., *Incidence, aetiology and epidemiology of uterine fibroids.* Best Pract Res Clin Obstet Gynaecol, 2008. **22**(4): p. 571-88.
148. Moss, J.G., et al., *Randomised comparison of uterine artery embolisation (UAE) with surgical treatment in patients with symptomatic uterine fibroids (REST trial): 5-year results.* Bjog, 2011. **118**(8): p. 936-44.
149. Bourdrez, P., M.Y. Bongers, and B.W. Mol, *Treatment of dysfunctional uterine bleeding: patient preferences for endometrial ablation, a levonorgestrel-releasing intrauterine device, or hysterectomy.* Fertil Steril, 2004. **82**(1): p. 160-6, quiz 265.
150. Pitter, M.C. and C. Simmonds, *The impact of different surgical modalities for hysterectomy on satisfaction and patient reported outcomes.* 2014. **3**(3): p. e11.
151. WL, A., *Removal of fibrous tumour of the uterus.* Am J Med Sci, 1845. **11**: p. 309-35
152. WA, A., *Myomectomy.* Med Press & Circular, 1889. **14**: p. 47.
153. Machupalli S, N.E., Mukherjee TK, Reilly KD, *Abdominal Myomectomy Increases Fertility Outcome.* Gynecol Obste, 2013. **3**: p. 144.
154. Metwally, M., Y.C. Cheong, and A.W. Horne, *Surgical treatment of fibroids for subfertility.* Cochrane Database Syst Rev, 2012. **11**: p. Cd003857.
155. van der Kooij, S.M., W.M. Ankum, and W.J. Hehenkamp, *Review of nonsurgical/minimally invasive treatments for uterine fibroids.* Curr Opin Obstet Gynecol, 2012. **24**(6): p. 368-75.
156. Tinelli, A., et al., *Laparoscopic myomectomy focusing on the myoma pseudocapsule: technical and outcome reports.* Hum Reprod, 2012. **27**(2): p. 427-35.
157. Sizzi, O., et al., *Italian multicenter study on complications of laparoscopic myomectomy.* J Minim Invasive Gynecol, 2007. **14**(4): p. 453-62.
158. Bernardi, T.S., et al., *Laparoscopic myomectomy: a 6-year follow-up single-center cohort analysis of fertility and obstetric outcome measures.* Arch Gynecol Obstet, 2014. **290**(1): p. 87-91.

159. Parker, W.H., et al., *Risk factors for uterine rupture after laparoscopic myomectomy.* J Minim Invasive Gynecol, 2010. **17**(5): p. 551-4.
160. Desai, P. and P. Patel, *Fibroids, infertility and laparoscopic myomectomy.* J Gynecol Endosc Surg, 2011. **2**(1): p. 36-42.
161. Gambadauro, P., J. Gudmundsson, and R. Torrejon, *Intrauterine Adhesions following Conservative Treatment of Uterine Fibroids.* Obstet Gynecol Int, 2012. **2012**: p. 853269.
162. Bougie, O., et al., *Treatment of Asherman's Syndrome in an Outpatient Hysteroscopy Setting.* J Minim Invasive Gynecol, 2014.
163. Bosteels, J., et al., *Hysteroscopy for treating subfertility associated with suspected major uterine cavity abnormalities.* Cochrane Database Syst Rev, 2013. **1**: p. Cd009461.
164. Sinha, R., et al., *Robotic surgery in gynecology.* J Minim Access Surg, 2015. **11**(1): p. 50-9.
165. Advincula, A.P. and A. Song, *Endoscopic management of leiomyomata.* Semin Reprod Med, 2004. **22**(2): p. 149-55.
166. Nezhat, C., et al., *Robotic-assisted laparoscopic myomectomy compared with standard laparoscopic myomectomy--a retrospective matched control study.* Fertil Steril, 2009. **91**(2): p. 556-9.
167. Pitter, M.C., et al., *Pregnancy outcomes following robot-assisted myomectomy.* Hum Reprod, 2013. **28**(1): p. 99-108.
168. Higham, J.M., P.M. O'Brien, and R.W. Shaw, *Assessment of menstrual blood loss using a pictorial chart.* Br J Obstet Gynaecol, 1990. **97**(8): p. 734-9.
169. Noyes, R.W. and J.O. Haman, *Accuracy of endometrial dating; correlation of endometrial dating with basal body temperature and menses.* Fertil Steril, 1953. **4**(6): p. 504-17.
170. Critchley, H.O. and P.T. Saunders, *Hormone receptor dynamics in a receptive human endometrium.* Reprod Sci, 2009. **16**(2): p. 191-9.
171. Roongsitthichai, A., et al., *Expression of cyclooxygenase-2 in the endometrium of gilts with different stages of endometritis.* J Vet Med Sci, 2011. **73**(11): p. 1425-31.
172. Fedchenko, N. and J. Reifenrath, *Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review.* Diagn Pathol, 2014. **9**: p. 221.
173. Obuchowski, N.A., *How many observers are needed in clinical studies of medical imaging?* AJR Am J Roentgenol, 2004. **182**(4): p. 867-9.
174. Rupp, G.M. and J. Locker, *Purification and analysis of RNA from paraffin-embedded tissues.* Biotechniques, 1988. **6**(1): p. 56-60.
175. Ribeiro-Silva, A., H. Zhang, and S.S. Jeffrey, *RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer samples: a comparison of column purification and magnetic bead-based technologies.* BMC Mol Biol, 2007. **8**: p. 118.
176. Specht, K., et al., *Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin-embedded tumor tissue.* Am J Pathol, 2001. **158**(2): p. 419-29.
177. Broekmans, F.J., et al., *Quantitative MRI of uterine leiomyomas during triptorelin treatment: reproducibility of volume assessment and predictability of treatment response.* Magn Reson Imaging, 1996. **14**(10): p. 1127-35.
178. Koga, K., et al., *Demonstration of angiogenin in human endometrium and its enhanced expression in endometrial tissues in the secretory phase and the decidua.* J Clin Endocrinol Metab, 2001. **86**(11): p. 5609-14.
179. Ferrara, N., *Vascular endothelial growth factor: basic science and clinical progress.* Endocr Rev, 2004. **25**(4): p. 581-611.
180. Furukawa, Y., et al., *The production of vascular endothelial growth factor and metalloproteinase via protease-activated receptor in human endometrial stromal cells.* Fertil Steril, 2009. **91**(2): p. 535-41.
181. Fan, X., et al., *VEGF blockade inhibits angiogenesis and reepithelialization of endometrium.* Faseb j, 2008. **22**(10): p. 3571-80.
182. Robinson, C.J. and S.E. Stringer, *The splice variants of vascular endothelial growth factor (VEGF) and their receptors.* J Cell Sci, 2001. **114**(Pt 5): p. 853-65.

183. Ferrara, N., et al., *The vascular endothelial growth factor family of polypeptides.* J Cell Biochem, 1991. **47**(3): p. 211-8.
184. Bakos, O., O. Lundkvist, and T. Bergh, *Transvaginal sonographic evaluation of endometrial growth and texture in spontaneous ovulatory cycles--a descriptive study.* Hum Reprod, 1993. **8**(6): p. 799-806.
185. Krikun, G., F. Schatz, and C.J. Lockwood, *Endometrial angiogenesis: from physiology to pathology.* Ann N Y Acad Sci, 2004. **1034**: p. 27-35.
186. Coudyzer, P., et al., *Hypoxia is not required for human endometrial breakdown or repair in a xenograft model of menstruation.* Faseb j, 2013. **27**(9): p. 3711-9.
187. Lockwood, C.J., *Mechanisms of normal and abnormal endometrial bleeding.* Menopause, 2011. **18**(4): p. 408-11.
188. Khan, K.N., et al., *Changes in tissue inflammation, angiogenesis and apoptosis in endometriosis, adenomyosis and uterine myoma after GnRH agonist therapy.* Human Reproduction, 2010. **25**(3): p. 642-653.
189. Vu, K., et al., *Cellular proliferation, estrogen receptor, progesterone receptor, and bcl-2 expression in GnRH agonist-treated uterine leiomyomas.* Human Pathology, 1998. **29**(4): p. 359-363.
190. Di Lieto, A., et al., *Preoperative administration of GnRH-a plus tibolone to premenopausal women with uterine fibroids: evaluation of the clinical response, the immunohistochemical expression of PDGF, bFGF and VEGF and the vascular pattern.* Steroids, 2005. **70**(2): p. 95-102.
191. Smith, S.K., et al., *Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding.* Br J Obstet Gynaecol, 1981. **88**(4): p. 434-42.
192. Tong, B.J., et al., *Heightened expression of cyclooxygenase-2 and peroxisome proliferator-activated receptor-delta in human endometrial adenocarcinoma.* Neoplasia, 2000. **2**(6): p. 483-90.
193. Wu, K.K., H.H. Cheng, and T.C. Chang, *5-methoxyindole metabolites of L-tryptophan: control of COX-2 expression, inflammation and tumorigenesis.* J Biomed Sci, 2014. **21**: p. 17.
194. Loftin, C.D., H.F. Tiano, and R. Langenbach, *Phenotypes of the COX-deficient mice indicate physiological and pathophysiological roles for COX-1 and COX-2.* Prostaglandins Other Lipid Mediat, 2002. **68-69**: p. 177-85.
195. Lim, H. and S.K. Dey, *Prostaglandin E2 receptor subtype EP2 gene expression in the mouse uterus coincides with differentiation of the luminal epithelium for implantation.* Endocrinology, 1997. **138**(11): p. 4599-606.
196. Lim, H., et al., *Multiple female reproductive failures in cyclooxygenase 2-deficient mice.* Cell, 1997. **91**(2): p. 197-208.
197. Davis, B.J., et al., *Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin-1beta.* Endocrinology, 1999. **140**(6): p. 2685-95.
198. Jabbour, H.N., et al., *Prostaglandin receptors are mediators of vascular function in endometrial pathologies.* Mol Cell Endocrinol, 2006. **252**(1-2): p. 191-200.
199. Uotila, P.J., R.U. Erkkola, and P.J. Klemi, *The expression of cyclooxygenase-1 and -2 in proliferative endometrium and endometrial adenocarcinoma.* Ann Med, 2002. **34**(6): p. 428-33.
200. Sugino, N., et al., *Withdrawal of ovarian steroids stimulates prostaglandin F2alpha production through nuclear factor-kappaB activation via oxygen radicals in human endometrial stromal cells: potential relevance to menstruation.* J Reprod Dev, 2004. **50**(2): p. 215-25.
201. Milne, S.A., et al., *Expression, localization, and signaling of PGE(2) and EP2/EP4 receptors in human nonpregnant endometrium across the menstrual cycle.* J Clin Endocrinol Metab, 2001. **86**(9): p. 4453-9.
202. Giatromanolaki, A., et al., *Differential assessment of angiogenic activity and of vascular survival ability (VSA) in breast cancer.* Clin Exp Metastasis, 2002. **19**(8): p. 673-9.

203. Han, H., et al., *Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis.* Ann Surg Oncol, 2001. **8**(1): p. 72-9.
204. Goteri, G., et al., *Expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1alpha (HIF-1alpha), and microvessel density in endometrial tissue in women with adenomyosis.* Int J Gynecol Pathol, 2009. **28**(2): p. 157-63.
205. Hlatky, L., P. Hahnfeldt, and J. Folkman, *Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us.* J Natl Cancer Inst, 2002. **94**(12): p. 883-93.
206. Hickey, M., et al., *A longitudinal study of changes in endometrial microvascular density in Norplant implant users.* Contraception, 1999. **59**(2): p. 123-9.
207. Rogers, P.A., C.L. Au, and B. Affandi, *Endometrial microvascular density during the normal menstrual cycle and following exposure to long-term levonorgestrel.* Hum Reprod, 1993. **8**(9): p. 1396-404.
208. Czekierdowski, A., et al., *Microvessel density assessment in benign and malignant endometrial changes.* J Physiol Pharmacol, 2008. **59 Suppl 4**: p. 45-51.
209. Tan, G., et al., *Study of the impact of uterine artery embolization (UAE) on endometrial microvessel density (MVD) and angiogenesis.* Cardiovasc Intervent Radiol, 2013. **36**(4): p. 1079-85.
210. Scholzen, T. and J. Gerdes, *The Ki-67 protein: from the known and the unknown.* J Cell Physiol, 2000. **182**(3): p. 311-22.
211. Gerdes, J., et al., *Growth fractions in malignant non-Hodgkin's lymphomas (NHL) as determined in situ with the monoclonal antibody Ki-67.* Hematol Oncol, 1984. **2**(4): p. 365-71.
212. Truskinovsky, A.M., B. Lifschitz-Mercer, and B. Czernobilsky, *Hyperplasia and carcinoma in secretory endometrium: a diagnostic challenge.* Int J Gynecol Pathol, 2014. **33**(2): p. 107-13.
213. Konstantinos, K., et al., *Expression of Ki-67 as proliferation biomarker in imprint smears of endometrial carcinoma.* Diagn Cytopathol, 2013. **41**(3): p. 212-7.
214. Germeyer, A., et al., *Changes in cell proliferation, but not in vascularisation are characteristic for human endometrium in different reproductive failures--a pilot study.* Reprod Biol Endocrinol, 2010. **8**: p. 67.
215. Marusak, R.A., Z.A. Radi, and L. Obert, *Expression of Ki-67 in the uterus during various stages of the estrous cycle in rats.* Exp Toxicol Pathol, 2007. **59**(3-4): p. 151-5.
216. Khaund, A., et al., *Evaluation of the effect of uterine artery embolisation on menstrual blood loss and uterine volume.* Bjog, 2004. **111**(7): p. 700-5.
217. T Justin Clark, J.K.G., *Endometrial sampling of gynaecological pathology.* The Obstetrician and Gynaecologist, 2002. **4**: p. 169-174.
218. St-Louis, I., et al., *Expression of COX-1 and COX-2 in the endometrium of cyclic, pregnant and in a model of pseudopregnant rats and their regulation by sex steroids.* Reprod Biol Endocrinol, 2010. **8**: p. 103.
219. Ma, Y., et al., *[Targeted interruption of COX-2 gene by siRNA inhibits the expression of VEGF, MMP-9, the activity of COX-2 and stimulates the apoptosis in eutopic, ectopic endometrial stromal cells of women with endometriosis].* Zhonghua Fu Chan Ke Za Zhi, 2015. **50**(10): p. 770-6.
220. Walker, W.J. and J.P. Pelage, *Uterine artery embolisation for symptomatic fibroids: clinical results in 400 women with imaging follow up.* Bjog, 2002. **109**(11): p. 1262-72.
221. JA, S., *The blood supply of uterine myomata.* Surgical Gynecology, 1912. **14**: p. 215-234.
222. Gomez-Jorge, J., et al., *Uterine artery anatomy relevant to uterine leiomyomata embolization.* Cardiovasc Intervent Radiol, 2003. **26**(6): p. 522-7.
223. Pron, G., et al., *The Ontario Uterine Fibroid Embolization Trial. Part 2. Uterine fibroid reduction and symptom relief after uterine artery embolization for fibroids.* Fertil Steril, 2003. **79**(1): p. 120-7.

224. Murase, E., et al., *Uterine leiomyomas: histopathologic features, MR imaging findings, differential diagnosis, and treatment.* Radiographics, 1999. **19**(5): p. 1179-97.
225. Hricak, H., et al., *Uterine leiomyomas: correlation of MR, histopathologic findings, and symptoms.* Radiology, 1986. **158**(2): p. 385-91.
226. Pelage, J.P., et al., *Uterine fibroid tumors: long-term MR imaging outcome after embolization.* Radiology, 2004. **230**(3): p. 803-9.
227. Firouznia, K., et al., *Uterine artery embolization in 101 cases of uterine fibroids: do size, location, and number of fibroids affect therapeutic success and complications?* Cardiovasc Intervent Radiol, 2008. **31**(3): p. 521-6.
228. Koesters, C., et al., *Uterine artery embolization in single symptomatic leiomyoma: do anatomical imaging criteria predict clinical presentation and long-term outcome?* Acta Radiol, 2014. **55**(4): p. 441-9.
229. Parthipun, A.A., et al., *Does size really matter? Analysis of the effect of large fibroids and uterine volumes on complication rates of uterine artery embolisation.* Cardiovasc Intervent Radiol, 2010. **33**(5): p. 955-9.
230. Katsumori, T., K. Nakajima, and T. Mihara, *Is a large fibroid a high-risk factor for uterine artery embolization?* AJR Am J Roentgenol, 2003. **181**(5): p. 1309-14.
231. Berczi, V., et al., *Safety and Effectiveness of UFE in Fibroids Larger than 10 cm.* Cardiovasc Intervent Radiol, 2015.
232. Smeets, A.J., et al., *Uterine artery embolization in patients with a large fibroid burden: long-term clinical and MR follow-up.* Cardiovasc Intervent Radiol, 2010. **33**(5): p. 943-8.
233. Spielmann, A.L., et al., *Comparison of MRI and sonography in the preliminary evaluation for fibroid embolization.* AJR Am J Roentgenol, 2006. **187**(6): p. 1499-504.
234. Naguib, N.N., et al., *Leiomyoma volume changes at follow-up after uterine artery embolization: correlation with the initial leiomyoma volume and location.* J Vasc Interv Radiol, 2010. **21**(4): p. 490-5.
235. Zlotnik, E., et al., *Predictive factors for pelvic magnetic resonance in response to arterial embolization of a uterine leiomyoma.* Clinics (Sao Paulo), 2014. **69**(3): p. 185-9.
236. Tropeano, G., et al., *Incidence and predictive factors for complications after uterine leiomyoma embolization.* Hum Reprod, 2014. **29**(9): p. 1918-24.
237. Verma, S.K., et al., *Submucosal fibroids becoming endocavitary following uterine artery embolization: risk assessment by MRI.* AJR Am J Roentgenol, 2008. **190**(5): p. 1220-6.
238. Redecha, M., Jr., et al., *Myoma expulsion after uterine artery embolization.* Arch Gynecol Obstet, 2009. **280**(6): p. 1023-4.
239. Hehenkamp, W.J., et al., *Myoma expulsion after uterine artery embolization: complication or cure?* Am J Obstet Gynecol, 2004. **191**(5): p. 1713-5.
240. Fleischer, A.C., et al., *Three-dimensional color Doppler sonography before and after fibroid embolization.* J Ultrasound Med, 2000. **19**(10): p. 701-5.
241. deSouza, N.M. and A.D. Williams, *Uterine arterial embolization for leiomyomas: perfusion and volume changes at MR imaging and relation to clinical outcome.* Radiology, 2002. **222**(2): p. 367-74.
242. Nikolaidis, P., et al., *Incidence of nonviable leiomyomas on contrast material-enhanced pelvic MR imaging in patients referred for uterine artery embolization.* J Vasc Interv Radiol, 2005. **16**(11): p. 1465-71.
243. Katsumori, T., et al., *Infarction of uterine fibroids after embolization: relationship between postprocedural enhanced MRI findings and long-term clinical outcomes.* Cardiovasc Intervent Radiol, 2008. **31**(1): p. 66-72.
244. Zimmermann, A., et al., *Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women.* BMC Womens Health, 2012. **12**: p. 6.
245. Wisot, A.L., K.M. Neimand, and A.H. Rosenthal, *Symptomatic myoma in a 13-year-old girl.* Am J Obstet Gynecol, 1969. **105**(4): p. 639-41.

246. Tsili, A.C., et al., *Fibromatous uterus in a 16-year-old girl: a case report.* Case Rep Med, 2010. **2010**: p. 932762.
247. Tanweer Karim, K.P., Anuradha Panchal, *Presentation and management of giant fibroid uterus in an adolescent girl.* Open Access Surgery, March 2010. **3**.
248. Fields, K.R. and L.S. Neinstein, *Uterine myomas in adolescents: case reports and a review of the literature.* J Pediatr Adolesc Gynecol, 1996. **9**(4): p. 195-8.
249. Van Voorhis, B.J., P.A. Romitti, and M.P. Jones, *Family history as a risk factor for development of uterine leiomyomas. Results of a pilot study.* J Reprod Med, 2002. **47**(8): p. 663-9.
250. Gabriel-Cox, K., et al., *Predictors of hysterectomy after uterine artery embolization for leiomyoma.* Am J Obstet Gynecol, 2007. **196**(6): p. 588.e1-6.
251. Sipola, P., et al., *Preinterventional quantitative magnetic resonance imaging predicts uterus and leiomyoma size reduction after uterine artery embolization.* J Magn Reson Imaging, 2010. **31**(3): p. 617-24.
252. Jha, R.C., et al., *Symptomatic fibroleiomyomata: MR imaging of the uterus before and after uterine arterial embolization.* Radiology, 2000. **217**(1): p. 228-35.
253. Tropeano, G., et al., *Long-term effects of uterine fibroid embolization on ovarian reserve: a prospective cohort study.* Fertil Steril, 2010. **94**(6): p. 2296-300.
254. Nalaboff, K.M., J.S. Pellerito, and E. Ben-Levi, *Imaging the endometrium: disease and normal variants.* Radiographics, 2001. **21**(6): p. 1409-24.
255. Kitao, K., et al., *The development of placenta increta following pelvic transcatheter artery embolization for postpartum hemorrhage.* Clin Exp Obstet Gynecol, 2009. **36**(1): p. 53-4.
256. Tropeano, G., et al., *Permanent amenorrhea associated with endometrial atrophy after uterine artery embolization for symptomatic uterine fibroids.* Fertil Steril, 2003. **79**(1): p. 132-5.
257. Ochi, J., et al., *Uterine changes during tamoxifen, toremifene, and other therapy for breast cancer: evaluation with magnetic resonance imaging.* Jpn J Radiol, 2010. **28**(6): p. 430-6.
258. Hauth, E., et al., *[MR Imaging of the pelvis in the diagnosis of the endometrium in breast cancer patients in tamoxifen therapy].* Rofo, 2006. **178**(3): p. 316-23.
259. Scheurig-Muenkler, C., et al., *Radiation Exposure During Uterine Artery Embolization: Effective Measures to Minimize Dose to the Patient.* Cardiovasc Intervent Radiol, 2014.
260. Spies, J.B., *Uterine artery embolization for fibroids: understanding the technical causes of failure.* J Vasc Interv Radiol, 2003. **14**(1): p. 11-4.
261. Razavi, M.K., et al., *Angiographic classification of ovarian artery-to-uterine artery anastomoses: initial observations in uterine fibroid embolization.* Radiology, 2002. **224**(3): p. 707-12.
262. Wolanske, K.A., et al., *Coil embolization of a tuboovarian anastomosis before uterine artery embolization to prevent nontarget particle embolization of the ovary.* J Vasc Interv Radiol, 2003. **14**(10): p. 1333-8.
263. Marx, M., et al., *Ovarian protection by occlusion of uteroovarian collateral vessels before uterine fibroid embolization.* J Vasc Interv Radiol, 2003. **14**(10): p. 1329-32.
264. Stoelinga, B., et al., *The estimated volume of the fibroid uterus: a comparison of ultrasound and bimanual examination versus volume at MRI or hysterectomy.* Eur J Obstet Gynecol Reprod Biol, 2015. **184**: p. 89-96.
265. van der Kooij, S.M., et al., *Uterine artery embolization vs hysterectomy in the treatment of symptomatic uterine fibroids: 5-year outcome from the randomized EMMY trial.* Am J Obstet Gynecol, 2010. **203**(2): p. 105.e1-13.
266. Spies, J.B., et al., *The FIBROID Registry: symptom and quality-of-life status 1 year after therapy.* Obstet Gynecol, 2005. **106**(6): p. 1309-18.
267. Spies, J.B., et al., *Complications after uterine artery embolization for leiomyomas.* Obstet Gynecol, 2002. **100**(5 Pt 1): p. 873-80.

268. Martin, J., K. Bhanot, and S. Athreya, *Complications and reinterventions in uterine artery embolization for symptomatic uterine fibroids: a literature review and meta analysis.* Cardiovasc Intervent Radiol, 2013. **36**(2): p. 395-402.
269. Leonhardt, H., A. Aziz, and L. Lonn, *Post-embolization syndrome and complete expulsion of a leiomyoma after uterine artery embolization.* Acta Obstet Gynecol Scand, 2005. **84**(3): p. 303-5.
270. Spies, J.B., *Recovery after uterine artery embolization: understanding and managing short-term outcomes.* J Vasc Interv Radiol, 2003. **14**(10): p. 1219-22.
271. Crea, F., et al., *Role of adenosine in pathogenesis of anginal pain.* Circulation, 1990. **81**(1): p. 164-72.
272. Hald, K., et al., *Uterine artery embolization versus laparoscopic occlusion of uterine arteries for leiomyomas: long-term results of a randomized comparative trial.* J Vasc Interv Radiol, 2009. **20**(10): p. 1303-10; quiz 1311.
273. Mehta, H., et al., *Review of readmissions due to complications from uterine fibroid embolization.* Clin Radiol, 2002. **57**(12): p. 1122-4.
274. Rajan, D.K., et al., *Risk of intrauterine infectious complications after uterine artery embolization.* J Vasc Interv Radiol, 2004. **15**(12): p. 1415-21.
275. Shlansky-Goldberg, R.D., et al., *Outcomes following fibroid expulsion after uterine artery embolization.* J Vasc Interv Radiol, 2011. **22**(11): p. 1586-93.
276. Radeleff, B., et al., *Expulsion of dominant submucosal fibroids after uterine artery embolization.* Eur J Radiol, 2010. **75**(1): p. e57-63.
277. Laverge, F., et al., *Spontaneous expulsion of three large fibroids after uterine artery embolization.* Fertil Steril, 2003. **80**(2): p. 450-2.
278. Preutthipan, S. and Y. Herabutya, *Vaginal misoprostol for cervical priming before operative hysteroscopy: a randomized controlled trial.* Obstet Gynecol, 2000. **96**(6): p. 890-4.
279. Ahmad, A., et al., *Uterine artery embolization treatment of uterine fibroids: effect on ovarian function in younger women.* J Vasc Interv Radiol, 2002. **13**(10): p. 1017-20.
280. Bergman, R., *An anatomy digital library - Uterine Artery.* Illustrated Encyclopedia of Human Anatomic Variation.
281. Lai, A.C., et al., *Sexual dysfunction after uterine artery embolization.* J Vasc Interv Radiol, 2000. **11**(6): p. 755-8.
282. *Fatal nontarget embolization via an intrafibroid arterial venous fistula during uterine fibroid embolization.* J Vasc Interv Radiol, 2009. **20**(3): p. 419-20.
283. Nikolic, B., et al., *Changes in blood coagulation markers associated with uterine artery embolization for leiomyomata.* J Vasc Interv Radiol, 2003. **14**(9 Pt 1): p. 1147-53.
284. Lanocita, R., *A fatal complication of percutaneous transcatheter embolization for treatment of uterine fibroids.* paper presented at : Society of Minimally Invasive Therapy/Centre for Innovative Minimmally Invasive Therapy, 11th international Conference, September 16-18, 1999.
285. Hamoda, H., P. Tait, and D.K. Edmonds, *Fatal pulmonary embolus after uterine artery fibroid embolisation.* Cardiovasc Intervent Radiol, 2009. **32**(5): p. 1080-2.
286. Czeyda-Pommersheim, F., et al., *Venous thromboembolism after uterine fibroid embolization.* Cardiovasc Intervent Radiol, 2006. **29**(6): p. 1136-40.
287. Freed, M.M. and J.B. Spies, *Uterine artery embolization for fibroids: a review of current outcomes.* Semin Reprod Med, 2010. **28**(3): p. 235-41.
288. Tropeano, G., S. Amoroso, and G. Scambia, *Non-surgical management of uterine fibroids.* Hum Reprod Update, 2008. **14**(3): p. 259-74.
289. Maresh, M.J., et al., *The VALUE national hysterectomy study: description of the patients and their surgery.* Bjog, 2002. **109**(3): p. 302-12.
290. Vashisht, A., et al., *Fatal septicaemia after fibroid embolisation.* Lancet, 1999. **354**(9175): p. 307-8.

291. de Blok, S., et al., *Fatal sepsis after uterine artery embolization with microspheres.* J Vasc Interv Radiol, 2003. **14**(6): p. 779-83.
292. Le Bihan, D., et al., *Diffusion MR imaging: clinical applications.* AJR Am J Roentgenol, 1992. **159**(3): p. 591-9.
293. Mannelli, L., et al., *Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver.* AJR Am J Roentgenol, 2009. **193**(4): p. 1044-52.
294. Tsien, C., Y. Cao, and T. Chenevert, *Clinical applications for diffusion magnetic resonance imaging in radiotherapy.* Semin Radiat Oncol, 2014. **24**(3): p. 218-26.
295. Bammer, R., *Basic principles of diffusion-weighted imaging.* Eur J Radiol, 2003. **45**(3): p. 169-84.
296. Hein, P.A., et al., *Diffusion-weighted magnetic resonance imaging for monitoring diffusion changes in rectal carcinoma during combined, preoperative chemoradiation: preliminary results of a prospective study.* Eur J Radiol, 2003. **45**(3): p. 214-22.
297. Liapi, E., et al., *Assessment of response of uterine fibroids and myometrium to embolization using diffusion-weighted echoplanar MR imaging.* J Comput Assist Tomogr, 2005. **29**(1): p. 83-6.
298. Hecht, E.M., et al., *Diffusion-weighted imaging for prediction of volumetric response of leiomyomas following uterine artery embolization: a preliminary study.* J Magn Reson Imaging, 2011. **33**(3): p. 641-6.
299. Faye, N., et al., *Diffusion-weighted imaging for evaluation of uterine arterial embolization of fibroids.* Magn Reson Med, 2013. **70**(6): p. 1739-47.
300. Stejskal EO, T.J., *Spin diffusion measurements: spinecho in the presence of a time dependent field gradient.* Journcal of Chemical Physics, 1965(42): p. 288-92.
301. Muir, K.W., et al., *Imaging of acute stroke.* Lancet Neurol, 2006. **5**(9): p. 755-68.
302. Whittaker, C.S., et al., *Diffusion-weighted MR imaging of female pelvic tumors: a pictorial review.* Radiographics, 2009. **29**(3): p. 759-74; discussion 774-8.
303. Ananthakrishnan, G., et al., *Diffusion-weighted imaging in uterine artery embolisation: do findings correlate with contrast enhancement and volume reduction?* Br J Radiol, 2012. **85**(1019): p. e1046-50.
304. Rees, M.C., *Role of menstrual blood loss measurements in management of complaints of excessive menstrual bleeding.* Br J Obstet Gynaecol, 1991. **98**(3): p. 327-8.
305. Higham, J.M. and R.W. Shaw, *Clinical associations with objective menstrual blood volume.* Eur J Obstet Gynecol Reprod Biol, 1999. **82**(1): p. 73-6.
306. Hallberg, L., et al., *Menstrual blood loss--a population study. Variation at different ages and attempts to define normality.* Acta Obstet Gynecol Scand, 1966. **45**(3): p. 320-51.
307. Edmonds, D.K., *Dewhurst's Textbook of Obstetrics and Gynaecology.* 2007(Chapter 42): p. 534.
308. Coulter, A., V. Peto, and C. Jenkinson, *Quality of life and patient satisfaction following treatment for menorrhagia.* Fam Pract, 1994. **11**(4): p. 394-401.
309. Wang, W., et al., *Iron deficiency and fatigue in adolescent females with heavy menstrual bleeding.* Haemophilia, 2013. **19**(2): p. 225-30.
310. Olive, D.L. and E.A. Pritts, *Fibroids and reproduction.* Semin Reprod Med, 2010. **28**(3): p. 218-27.
311. Hallberg, L. and L. Nilsson, *DETERMINATION OF MENSTRUAL BLOOD LOSS.* Scand J Clin Lab Invest, 1964. **16**: p. 244-8.
312. van Eijkeren, M.A., et al., *The alkaline hematin method for measuring menstrual blood loss--a modification and its clinical use in menorrhagia.* Eur J Obstet Gynecol Reprod Biol, 1986. **22**(5-6): p. 345-51.
313. Deeny, M. and J.A. Davis, *Assessment of menstrual blood loss in women referred for endometrial ablation.* Eur J Obstet Gynecol Reprod Biol, 1994. **57**(3): p. 179-80.

314. Zakherah, M.S., et al., *Pictorial blood loss assessment chart in the evaluation of heavy menstrual bleeding: diagnostic accuracy compared to alkaline hematin.* Gynecol Obstet Invest, 2011. **71**(4): p. 281-4.
315. Larsen, L., K. Coyne, and K. Chwalisz, *Validation of the menstrual pictogram in women with leiomyomata associated with heavy menstrual bleeding.* Reprod Sci, 2013. **20**(6): p. 680-7.
316. Hald, K. and M. Lieng, *Assessment of periodic blood loss: interindividual and intraindividual variations of pictorial blood loss assessment chart registrations.* J Minim Invasive Gynecol, 2014. **21**(4): p. 662-8.
317. Schumacher, U., et al., *Estimation of menstrual blood loss volume based on menstrual diary and laboratory data.* BMC Womens Health, 2012. **12**: p. 24.
318. Wyatt, K.M., et al., *Determination of total menstrual blood loss.* Fertil Steril, 2001. **76**(1): p. 125-31.
319. Dasharathy, S.S., et al., *Menstrual bleeding patterns among regularly menstruating women.* Am J Epidemiol, 2012. **175**(6): p. 536-45.
320. Gudmundsdottir, B.R., et al., *Quantification of menstrual flow by weighing protective pads in women with normal, decreased or increased menstruation.* Acta Obstet Gynecol Scand, 2009. **88**(3): p. 275-9.
321. Higham, J.M., P.M. O'Brien, and R.W. Shaw, *Assessment of menstrual blood loss using a pictorial chart.* Br.J.Obstet.Gynaecol., 1990. **97**(8): p. 734-739.
322. McPherson, K., et al., *A randomised trial of treating fibroids with either embolisation or myomectomy to measure the effect on quality of life among women wishing to avoid hysterectomy (the FEMME study): study protocol for a randomised controlled trial.* Trials, 2014. **15**: p. 468.
323. Redecha, M., Jr., et al., *Pregnancy after uterine artery embolization for the treatment of myomas: a case series.* Arch Gynecol Obstet, 2013. **287**(1): p. 71-6.
324. Yong Li, Z.G., *Expressions of MVD, VEGF, Ki67 in residual prostate cancer after cryoablation.* Clinical Oncology and Cancer Research, 2011. **8**(1): p. 27-32.
325. RCOG/RCR, *Clinical recommendations on th euse of uterine artery embolisation (UAE) in the management of fibroids.* 2013.
326. Czuczwar, P., et al., *Predicting the results of uterine artery embolization: correlation between initial intramural fibroid volume and percentage volume decrease.* Prz Menopauzalny, 2014. **13**(4): p. 247-52.
327. Tamai, K., et al., *Diffusion-weighted MR imaging of uterine endometrial cancer.* J Magn Reson Imaging, 2007. **26**(3): p. 682-7.
328. Chua, G.C., et al., *Comparison of particle penetration with non-spherical polyvinyl alcohol versus trisacryl gelatin microspheres in women undergoing premyomectomy uterine artery embolization.* Clin Radiol, 2005. **60**(1): p. 116-22.
329. Kovacs, P., et al., *The effect of endometrial thickness on IVF/ICSI outcome.* Hum Reprod, 2003. **18**(11): p. 2337-41.