STUDIES OF FALLOPIAN TUBE PATENCY

IN THE COW

BY

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SUMMARY

On gross examination of 308 fresh female bovine genital tracts abnormalities which involved the oviducts were adhesions (20.5%), paratubal cysts (4.87%) and hydrosalpinx (1.62%). One hundred and eighty of these were studied in more detail and in 3 both oviducts were blocked whereas unilateral blockage was found in 6.

Insufflation studies on apparently normal tracts showed a wide variation of pressures at which air escaped from the oviducts. The main area of resistance to the passage of air was the isthmus. Endometrial rupture occurred in 31% of the tracts during transuterine insufflation of the oviducts although in 98% of these both oviducts were patent. Subsequent passage of air down the oviducts required significantly higher pressures.

The phenosulphonphthalein dye test was studied to determine its clinical application in cattle. This involved instillation of the dye into the body of the uterus via the cervix and detecting its passage through the oviducts into the peritoneum by its presence in the urine. When the dye was detected within 15-30 minutes both oviducts were patent. The stage of the oestrous cycle did not have any obvious effects on the test. In some cases with delay in appearance of the dye in urine, on repeating the test two or three times there was a reduction in the time of appearance of the dye. If after 3 tests the dye did not appear in the urine until about 45-60 minutes, one oviduct was occluded and if it was not detected by 120 minutes, both oviducts were occluded, these findings being confirmed both on post-mortem examination and by carrying out

oviduct ligation in 2 animals.

The test did not cause obvious damage to the epithelium lining of the uterus or oviducts and resulted in an almost complete emptying of oviduct lumenal contents. The carcase value was not lowered and the future fertility did not appear to be reduced since 4 of the 5 animals which were inseminated conceived to first insemination and were all diagnosed pregnant at 2½ months.

Endoscopy demonstrated that the dye passed through the oviducts. In most cases the entire genital tract could be seen from either left or right flank, the latter being an easier method with better visualisation. In fat animals both left and right flank approaches were necessary for complete visualisation.

INTRODUCTION

The oviduct is of functional importance in many ways. It is involved with the process of fertilisation, which occurs in the lower portion of the ampulla near the ampullary isthmic junctions (AIJ) and with the subsequent transport of the developing zygote until it reaches the uterus (McLaren, 1974; Blair and Beck, 1975; Hafez, 1974; El Banna and Hafez, 1970a).

In the cow the oviduct can easily be identified in the post-mortem specimen as a tortuous, loosely coiled tube forming a large loop at the ovarian end and becoming increasingly firm, in the distal half. In the cow the oviducts are about 25-35 cm long and extend from the fimbriated portion of the infundibulum at the ovarian end to the uterotubal junction (UTJ) at the tip of the uterine horn. Each oviduct runs along the free margin of the broad ligament, the outer peritineal layer of which loops upwards and around the oviduct. This double peritoneal fold, the mesosalpinx, has variable amounts of fat in which the oviduct is imbedded. The mesosalpinx and the mesotubarium superius are extensive at the large loop of the oviduct at the ovarian end forming a deep peritoneal sac, the ovarian bursa, which hangs free from the ovary.

For descriptive purposes the oviduct in the cow can be divided into four parts, viz, the infundibulum, the ampulla, the isthmus and the uterotubal junction.

1. <u>The infundibulum</u> - the funnel shaped ovarian end of the oviduct has a wide thin wa .ed fimbriated edge which at one point

is attached to the ovary and at another associated with the mesosalpinx, forming a part of the free edge of the ovarian bursa. The open ovarian end of the infundibulum is directed inwards towards the ovary within the ovarian bursa. The diameter of the oviduct is greatest in the infundibulum, where the mucosa is thrown into longitudinal folds with prominent secondary branches, and the tips of some of the primary folds extending past the centre of the lumen. In this region the innermost layer lining the lumen is a pseudostratified epithelium with a predominance of ciliated cells over the mucous secreting cells. Cilia are present throughout the length of the oviduct but are most numerous in the infundibulum.

At ovulation, which in the cow occurs 13-16 hours after the end of oestrus (Foote, 1974), the fimbriated end of the oviduct receives the ovum by a mechanism which is not yet clear. El Banna et al., (1970a) suggested that the ovum transport from the fimbriated end to approximately a quarter way down the oviduct is affected primarily by the action of cilia which beat in a downward direction. Ovum transport through the infundibulum is very rapid. As early as 1940, Gerasimova, Potapova, Solovei and Hvatov stated that 6 hours after ovulation the ovum has travelled through one third of the oviduct. Jarosz (1959) reported similar findings. El Banna et al. (1970a) working with nulliparous heifers reported that some fertilised ova are already more than half way down the oviduct by 8-10 hours after ovulation, only one celled ova being found in the infundibulum.

The secretory cells of the infundibulum are more active in the follicular phase than at any other stage of the oestrous cycle (Nayak and Ellington, 1972). Cytoplasmic extrusions of

these secretory cells, often including nuclei and other cell organells and secretory granules, have been reported, but there is still a controversy as to the stage of the oestrous cycle when such extrusions are most prevalent (Lombard, Morgan and McNutt, 1950; Weeth and Herman, 1952; Wordinger, Dickey and Hill, 1976).

The ciliated cells in the infundibular region increase in height during oestrus and immediately afterwards (Lombard et al., 1950; McDaniel, Scalzi and Black, 1968). Stalheim, Gallagher and Deyoe (1975) recently demonstrated by scanning electron microscopy that the cilia in the oviduct were longer, more erect, more distinct and separate from one another during oestrus than in any other phase of the oestrous cycle.

2. Ampulla - This segment of the oviduct comprises approximately 2/3rds of the total length. At this point the oviduct tapers down from its widest end at the junction with the infundibulum towards the Ampullary Isthmic Junction (AIJ). The mucosal folds have a similar arrangement to those of the infundibulum. In the ampulla there is a predominance of the ciliated cells over non-ciliated mucus secreting cells in the epithelium lining the lumen, and the increase in the height of cells at oestrus and immediately afterwards is even more pronounced here than in the infundibulum (Lombard et al; 1950; McDaniel et al., 1968).

There is general agreement that the cytoplasmic extrusions, some found in the cells lining the upper part of the oviduct of the cow, are most prevalent in the upper part of the ampullary region and appear to be attached to the epithelial cells by thin strands of cytoplasm (Lombard et al., 1950; Weeth et al., 1952;

and Wordinger et al., 1976). However, in the lower ampullary region cytoplasmic extrusions are very rare at any stage of the cycle (Lombard et al., 1950; Weeth et al., 1952; McDaniel et al., 1968.

El Banna and Hafez (1970a) suggested that the ovum transport through the initial part of the ampulla is primarily by the action of the cilia beating downwards in a wavelike motion as in the infundibulum. Further progress of the ovum was mainly due to muscular contractions rather than ciliary action resulting in a 'hesitant advance of the ovum one segment at a time'.

In 1970b El Banna and Hafez slaughtered nulliparous Hereford cattle at various stages of the oestrous cycle, removed the oviducts from the genital tract, pinned them out in a paraffin lined enamel tray without straightening them and immersed them in AFA fixative (30% ethanol, 95%, 10% commercial formalin, 10% glacial acetic acid and 50% water) for 2 days. Sections were cut and only round sections were used indicating that they had been taken exactly at right angles to the lumen. Sections were projected at a known magnification and traced. The area of the lumen, mucosa, submucosa and muscular layer were then measured planimetrically. This work showed that the AIJ is probably located at a point 6/10th to 7/10th down the oviduct starting from the fimbriated end. The planimetric measurements also showed that this area of the oviduct has the lumen with the smallest surface area at all stages of the oestrous cycle. However, about 72 and 168 hours post-ovulation the surface area of the lumen at this part of the oviduct increased twofold as compared to that at oestrus.

El Banna and Hafez (1970b) suggested that the narrowing of the lumen of the AIJ may occur through circular muscle spasm and that presence or absence of oestrogens rather than progesterone may be responsible for sphincter-like contractions at various parts of the oviduct. They further suggested that the mucosal folds may control the lumen diameter by bringing about changes in the diameter of mucosal vessels and a change in the amount of interstitial fluid. Nayak, Ellington and Zimmerman (1974) reported that the lymphatic capillaries in the oviduct are more dilated during oestrus than at any other stage of the oestrous cycle. Likewise increase in the epithelium cell height at oestrus under the influence of oestrogen as compared with other stages of the oestrous cycle may be a contributing factor to the control of the lumen size.

Some ova are already in the ampulla near the AIJ 8-10 hours after ovulation. After this initial rapid descent the ovum remains in the ampulla near the AIJ for about 72 hours post-ovulation (El Banna and Hafez, 1970a).

3. The isthmus forms about 1/3rd of the total tubule length and is the narrowest segment of the tube with an external diameter of approximately 1-2 mm. The isthmus has a thick wall which is formed by a well developed circular muscle layer. The mucosa is thrown into longitudinal folds, but the tips of the folds do not extend past the centre of the lumen. The epithelium lining the lumen has a predominance of non-ciliated secretory cells over the ciliated cells which is the reverse of the situation in the ampulla and infundibulum. The change in the height of the cells shows a similar trend to that of the ampulla and infundibulum epithelium being highest at oestrus but the changes are less marked. The secretory cells in the

isthmus very rarely have cytoplasmic extrusion at any stage of the oestrous cycle (Lombard et al., 1950; Weeth et al., 1952; McDaniel et al., 1968).

The uterotubal junction (UTJ) is the area of transition where the isthmus joins the tip of the uterine horn. At the region of junction of isthmus and uterus there are no caruncles present within the endometrium and the mucosal folds of the isthmus disappear 1-3 mm from the joining. At the UTJ there is no intramural segment, the isthmus not projecting into the lumen of the uterus, as it does in some other species.

In the cow no anatomical barrier has been demonstrated at the UTJ and recent work has failed to substantiate the suggestion of the earlier workers that the UTJ acts as a valve resulting in ovum retention in the oviduct at this time.

A review of fallopian tube patency techniques

In assessing fertility in the bovine female, rectal palpation of the genital tract includes examination of the fallopian tubes. Such an examination, at best, only indicates gross abnormalities of these structures, and it is now generally accepted that some test or technique which could be applied in the living animal to indicate patency of the fallopian tubes in female cattle would be of benefit in the diagnosis of certain cases of infertility in this species.

Various tests for patency of oviducts in the human medicine have been carried out for many years now and some are at present used routinely as diagnostic tests in cases of infertility in women. Recently some of these methods have been tried in cattle.

The first test in humans involving transuterine insufflation of oviducts by gas was reported by Rubin (1920). The principle underlying this test is that when gas is passed under pressure through the cervix it will travel via the uterus and oviducts to escape at the ovarian open end of each oviduct into the peritoneal cavity. In his work Rubin used a manometer to record the fall in pressure indicating the escape of gas from the fallopian tubes. Various minor modifications of this initial method have been used, e.g. the use of kymographic tracings as a demonstration of gas having escaped (Sweeney, 1962).

However, despite the various modifications in an attempt to improve this test there are many reports of high incidences of unreliable results (Stallworthy, 1948; La Forge, 1951; Grant and Mackey, 1957).

One of the earliest reports in the literature of such tests in cattle was that of Williams (1925) who carried out the Rubin test in cattle using corbondioxide and checked the reproductive tract after slaughter. He reported fairly constant results. However, Williams discovered that the introduction of gas under very high pressure could cause rupture of the endometrium in cattle. He therefore never exceeded pressures of over 100 mm of mercury and reported that under these conditions the cattle did not suffer any ill effects.

Rowson (1942) carrying out the test in vitro reported 25% incidence of rupture of the endometrium. This rupture occurred at the attached border of the lesser curvature of the uterus before the escape of air via the oviduct. Spriggs (1945) carried out insufflation with air in 3 heifers and 2 cows using a Higginson's enema pump with a catheter inserted just through the internal os of the cervix. In 4 of these 5 animals subsequent post-mortem showed a small area of rupture of the endometrium had occurred midway along the lesser curvature of either the left or the right uterine horn with escape of air in between the double peritoneal layer of the broad ligament. He agreed with Rowson (1942) that this test was too dangerous to be of value in cattle.

Hanley (1953) tried a further modification of this test in cattle by a suitably altered cannula such as that used in this test in women. The cannula caused obturation of the internal os of the cervix and using it, Hanley claimed success with insufflation in cattle both in vivo and in vitro. He, however, reported that by using a low rate of gas inflow and avoiding a rapid increase in pressure patency was determining

accurately in those cows where pressure of only 70-80 mm Hg was required and that using this approach misinterpretation of pressure changes was reduced.

Hanley concluded that the maximum safe pressure at which it is possible to diagnose patency in at least one oviduct is 80 mm Hg, and that the margin of safety is very small, about 10 mm Hg as compared to about 100 mm Hg in women. He also reported cases of rupture of the uterus at the same site as Rowson (1942). Kawata and Koike (1959) took things a step further by including in this test simultaneous rectal palpation of the fimbriated end of the oviduct recording a fremitus-like sensation in normal cows as the gas escaped via the oviduct. However, in 58 cows and 2 heifers they reported some discrepancies between the results of the test and post-mortem findings. They also reported 17.9% cases of rupture of the endometrium. Von Busch (1962) also reported cases of uterine and oviductal rupture with this test.

Kowaliszyn (1969) carried out gas insufflation in 53 infertile cattle using a modification of Evers pump. It had an air filter and the catheter modified to obliterate the lumen of the cervix. The catheter was introduced not more than 10 cms deep into the cervix. He found that at a pressure of 250-300 mm Hg air passed along the oviduct into the peritoneum easily but exceeding this pressure would cause rupture of the oviducts. In 20 of the cattle tested there was resistance to the flow of gas suggesting occlusion of one or both of the oviducts. However, after intrauterine treatment of all the 20 cows with novocaine and cortisone, 9 became pregnant. In a further 3 animals, conception was achieved after a second ourse of insufflation and treatment.

Kowaliszyn suggested that insufflation benefited these cases of oviduct occlusion by removing blood or mucous clots from the affected oviducts.

From the various reports on the tubo-insufflation test there is obviously still some confusion as to the feasibility of the test in cattle as a diagnostic method.

Starch-iodine test

In 1954 McDonald devised a test to detect oviduct patency in cattle whereby sterile solution of starch injected intra-peritoneally is detected 2-3 days later in the vaginal swabs. The swabs are placed in small amounts of saline solution to which a few drops of Lugols iodine has been added. The presence of bluish-purple granules in this solution was examined under a microscope and indicates at least one patent oviduct. With this method, however, unilateral occlusion of the oviduct can be undetected.

As a modification of the above, Johari and Sharma (1964) used a glass pipette to collect the vaginal mucus. They suggested that the starch granules were metabolised in either the peritoneum or the genital tract and that the blue reaction recorded by McDonald was some contamination of cotton wool of the swabs used for collection of vaginal mucus.

Phenylsulfonphthalein test (P.S.P. test)

In 1948 Speck reported that when P.S.P. dye is injected into the uterus in women it passes via the oviducts, if patent, into the peritoneal cavity where it is readily absorbed and filtered by the kidneys and is excreted in the urine. Its presence in the urine can be detected by the change of colour to pink or red on alkalinising the urine by addition of sodium

hydroxide. When both oviducts are blocked this colour reaction does not occur. On the basis of his experiments he also reported that P.S.P. is only slowly absorbed if at all from the normal vaginal mucosa, endometrium and endosalpinx. He claimed that if the oviducts were patent dye should be demonstrated in the urine within 30 minutes of introduction of the dye into the uterus. Any increase in this time of appearance of the dye in the urine suggested abnormalities of one or both oviducts.

He got very accurate information in 16 women. In 3 of the 10 normal cases the result of subsequent hysterosalpingography substantiated the results. Likewise in 3 of the 7 cases with non-patency as indicated by the P.S.P. test subsequent surgery confirmed the diagnosis in all 3.

As this test proved simple to perform and quite reliable it rapidly gained popularity.

Gromadzki, Lukasik and Papierowski (1965), however, reported that in women the absolute accuracy was only 55%, false positive results arising possibly from the absorption of P.S.P. from the lining of hydrosalpinges, damaged endometrium or endosalpinx.

Thomas (1962) reviewing patency tests in women concluded that the P.S.P. test adds little to the information obtained by gas insufflation and hysterosalpingography.

Nevertheless, this test has been used for many years and is still used routinely in human medicine to determine the patency status of the oviduct.

One of the first reported uses of a dye test in cattle was that of Otel and Drume (1968) who carried out a test using indigo carmine instead of P.S.P. in 33 cows checking the urine for

the presence of the dye 2 hours after its instillation into the uterus. Post-mortem examination confirmed the 23 positive tests and 8 out of 10 negative tests, all of the 8 having salpingitis, the remaining 2 negative cases had patent oviducts. They concluded that this method of diagnosis is valuable but will miss some cases. Following this work Berchtold and Brummer (1968) investigated this test in 21 animals and comparing the results with the post-mortem findings had only 1 wrongly diagnosed case. They concluded that this test in cattle is probably more reliable than any other existing methods, and suggested the critical time for finding the colour reaction in urine to be 30 minutes after instilling the dye in the uterus which is in accordance with the recommendation of Speck (1948) for the test in women.

Dzhurova and Ushev (1974) found a very high incidence of inaccuracy. They reported that post-mortem revealed that in 12 cows with positive colour reaction, 8 had obstruction and 10 cows with negative test, 8 had 'normal' patency of the oviducts. They collected the urine samples at 30 and 90 minutes after instillation of the dye in uterus and used ammonium hydroxide to render it alkaline.

Von Schneider and Rüsch (1976) carried out this test in 10 animals on 37 occasions and found 33 positive and 4 questionable results. They repeated the test on 46 occasions on the same animals after bilateral ligation of the oviducts, and got 32 negative, 6 positive and 8 dubious tests. They suggested that the false positive results could arise from absorption of the dye via damaged endometrium and/or contamination of the urine by the dye from the genital tract. They further suggested that there was a possibility

of getting false negative results with animals in oestrus.

From the reports of the work already done it would appear that the P.S.P. test could have a future as a routine field diagnostic test for oviduct patency in cattle. However, as with all methods of testing oviduct patency, several aspects of the test in cattle require elucidation. It was decided to carry out an investigation of oviduct patency in cattle using endoscopy, histological and practical methods.

SECTION 1

[redacted] of:-

A. Gross abnormalities of bovine female genital tracts *in vitro* with particular attention to fallopian tubes.

B. Air insufflation of bovine female genital tracts *in vitro* with particular attention to fallopian tubes.

A.

Introduction

In an attempt to determine the incidence of blocked oviducts and the types of abnormalities of the oviduct which occur in cattle, it was decided to collect, at post-mortem, a random sample of genital tracts from female cattle and carry out gross examination of the oviducts.

Materials and methods

Immediately after slaughter, tracts were collected and the ovaries, oviducts and uterus were carefully examined macroscopically. Those which had apparent abnormalities of the oviducts were isolated. The presence of adhesions between the ovaries and bursa and the oviduct were looked for and recorded where present. At the same time obvious abnormalities such as hydrosalpinx and paratubal cysts were noted. Using a 20 ml syringe and inserting a 21 gauge hypodermic needle into the tip of each uterine horn and passing air, it was determined whether or not the tubes were blocked.

Results

TABLE 1

GROSS ABNORMALITIES IN THE OVIDUCTS OF THE BOVINE GENITAL TRACTS OBTAINED AT RANDOM

Total No. of Specimens	Adhesions		Hydrosalpinx		Para-tubal Cysts	Normal
	Unilateral	Bilateral	Unilateral	Bilateral		
308	51	12	2	3	15	225
%	16.6o	3.9o	0.65	0.97	4.87	73.05

Table 1 shows the results of the gross examination of bovine female genital tracts *in vitro*. Note that in 308 tracts examined 73.05% were apparently normal. Adhesions were the commonest abnormality.

TABLE 2

INCIDENCE OF BLOCKED OVIDUCTS *IN VITRO* IN BOVINE FEMALE GENITAL TRACTS

Total No. of Specimens	Blocked		Normal
	Unilateral	Bilateral	
180	6	3	171
%	3.33	1.7o	95.0

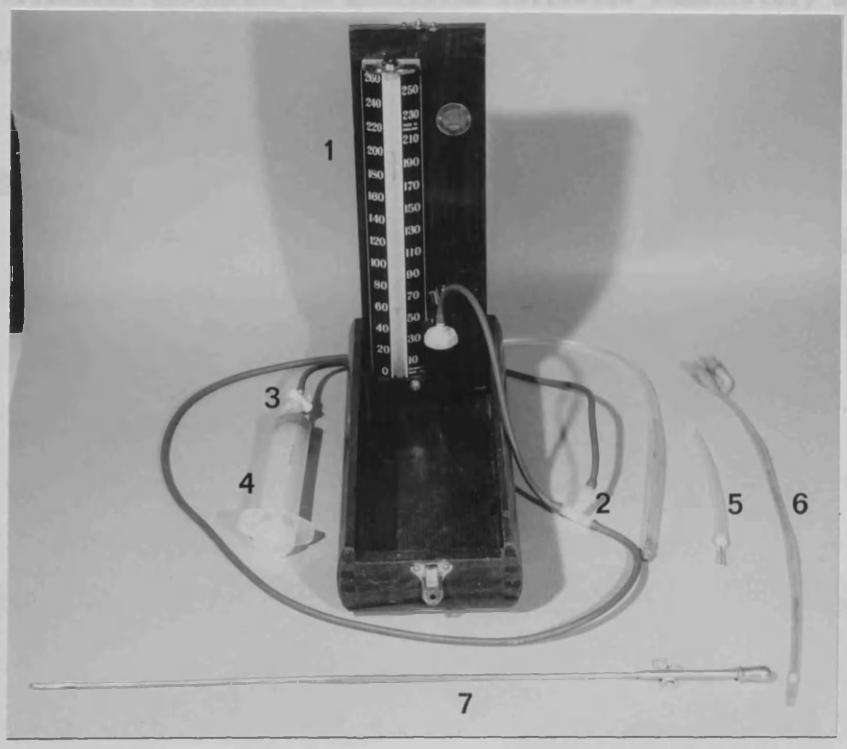
Table 2 shows the results of the studies for blockage of oviducts in 180 tracts. Only 1.7% had bilateral blockage, 3.33% unilateral blockage.

Discussion

From the results of the study described here, the percentage of abnormal oviducts in cattle would appear to be in the region of 25%. The largest percentage of abnormalities was not causing occlusion of the actual oviduct itself but was found in either its attachments at the ovarian end or in adnexa in the broad ligament. In the cases where there were adhesions these would obviously affect fertility, since in some of the cases the adhesions were so severe as to make it impossible for the fimbriated end of the oviducts to receive the ova at ovulation. Some of the adhesions alongside the length of the oviduct were increasing the tortuousness of the oviducts themselves and so could cause reduction of fertility by hindering the passage of both the ova and sperm through the oviduct.

Except in two cases where the cysts were embedded in the oviduct wall, the cysts were found at the fimbriated end or on the masosalpinx, and were not apparently causing any problem as far as the oviduct was concerned. Indeed actual occlusion of the oviduct was only demonstrated in this preliminary investigation in approximately 5% of the tracts examined, and of these the greatest percentage was unilateral. This fact might on first consideration indicate that abnormal patency of oviducts in cattle is not a grave problem. However, bearing in mind that the studies reported here were carried out in post-mortem specimens and realising the amount of investigation this has stimulated in the human patient, such findings must be viewed with care.

FIGURE 1



Equipment used in air insufflation technique

B.

Introduction

As an initial approach to the investigation of the patency of oviducts in adult cattle, it was decided to apply the air insufflation technique to genitalia collected immediately after slaughter from a random selection of female cattle.

Materials and methods

The equipment used for this study is shown in Fig. 1.

1. Mercury manometer (Blood Pressure Standard, Accson).
2. T system of tubes.
3. 3-way valve.
4. 50 ml. syringe.
5. A tube with a needle with grooves on the shaft and tip cut off.
6. Foley's self-retaining catheters.
7. Neilson's metal catheter.

Immediately after slaughter the genital tracts were dissected out on an enamel tray filled with water.

Method 1.

A Foley's catheter was introduced into the body of the uterus via the cervix. If difficulty was experienced in passing the Foley's catheter, the cervix was dilated by a Neilson's catheter prior to insertion of the Foley's catheter. Once the Foley's catheter was in situ, the cuff was blown to form an airtight seal.

The other end of the Foley's catheter was then attached to the tube C and the whole genital tract was immersed in a waterbath.

Air was pumped in slowly increasing the pressure 2-3 mm at a time until it was seen to escape via the ovarian end of the oviduct. This was indicated by bubbles of air escaping from the ovarian open end of the oviduct and a simultaneous fall in pressure on the manometer.

The maximum pressure registered on the manometer was recorded and the 3-way valve was then put in the closed position. This ensured that any fall in pressure registered on the manometer would be due to escape of air via the open ovarian end of the oviduct and nowhere else. The system was then left until no further air escaped and the new basal pressure recorded. This remained constant.

In those cases where the body of the uterus was small, air was passed up only one horn. Then the position of the catheter was readjusted so as to allow passage of air up the second horn and recordings taken.

Method 2

In [illegible] cases, subsequent to passing of the air in above method, the following was carried out. Once the basal pressure was reached the 3-way valve was still kept in the closed position. The mesotubarium superius was carefully dissected out with scissors with the minimum possible handling, taking care not to disturb the oviduct in any way. Any subsequent fall in pressure was recorded.

Method 3

In [illegible] genital tracts where the endometrium ruptured before the escape of air via the oviducts, uterine horns were cut approximately 5 cms from the utero-tubal junction (UTJ). Tube C was introduced in the uterine horn and held in place by a ligature

and air was passed up oviduct and the pressures recorded.

Method 4

In [illegible] genital tracts after passing air up the oviducts via the uterus, uterine horns were cut approximately 5 cms from the UIJ and air was passed down the oviducts. A needle was inserted into the oviduct for a distance of 1 to 2 cms and held in place by a ligature passed via small perforations made in the supporting mesentery on either side to avoid disturbing the normal position of the oviduct. The open end of the tube with the needle was then attached to tube C, air was pumped in slowly and the initial build up and the final basal pressures recorded.

Histology

A random sample of 6 genital tracts used in the insufflation study was selected.

All of the uteri were cut open and any abnormalities noted. A 2 cm square piece of both uterine horns was taken just anterior to the external bifurcation of the uterine horns.

Both oviducts were dissected free from their attachments and 1 cm of the fimbriated portion of the oviduct, 1 cm piece of the ampulla, 5 cms from the ovarian open end of the oviduct and 1 cm piece of isthmus 3 cms away from the UTJ were collected from both oviducts.

Preparation

All specimens were collected within 15 minutes after slaughter and fixed in 10% formol saline for at least 48 hours. They were then dehydrated through a series of upgraded alcohol and impregnated with colloidon and methyl benzoate. They were then cleared in amyl acetate and finally impregnated with paraffin wax.

TABLE 3

MAXIMUM AND MINIMUM READINGS OF AIR PRESSURES RECORDED WHEN PASSING AIR THROUGH THE OVIDUCTS FROM UTERINE END TOWARDS OVARY IN BOVINE GENITAL TRACTS IN VITRO

Specimen No.	Ovarian Findings		Registered pressures in mm Hg				Presence or absence of endometrial rupture	Pressure at which rupture occurred
			Left Oviduct		Right Oviduct			
	Left Ovary	Right Ovary	Max. mm Hg	Min. mm Hg	Max. mm Hg	Min. mm Hg		
1	-	, f	180	107	185	92	-	-
2	-	f CL	150	76	175	55	+	140
3		CL	110	55	140	80	-	-
	-	F	230	44	250	194	+	180
5	f CL	-	70	48	180	60	+	150
6	F	-	100	32	85	54	-	-
7	f	F, Ff, CL	200	62	150	100	+	143
8	f CL	F	142	48	110	54	+	100
9	-	C CL	118	50	138	45	-	-
10	Ff	-	98	52	110	75	-	-
11	-	f	180	57	160	30	-	-
12	-	f	120	57	127	80	-	-
13	f	CL	115	72	104	74	+	98
14	-	Ff	100	54	108	64	-	-
15	-	f	210	98	135	80	+	120
MEAN			141.53	60.80	143.80	75.80		133.00
STANDARD DEVIATION			47.91	20.03	41.90	37.51		29.24
STANDARD ERROR			12.37	5.17	10.81	9.68		71.05

KEY: CL = Corpus luteum over 2 cm in diameter: F = Follicle over 1.5 cm in diameter: f = Follicle over 1 cm and under 1.5 cm in diameter.

TABLE 4

AIR PRESSURES RECORDED WHEN PASSING AIR UP THE OVIDUCTS VIA THE UTERUS IN BOVINE GENITAL TRACTS IN VITRO - MAXIMUM, MINIMUM AND AFTER DISSECTING OUT MESOTUBARIUM SUPERIUS (3)

Specimen No.	Ovarian Findings		Registered pressures in mm Hg						Presence or absence of endometrial rupture	Pressure at which rupture occurred
			Left Oviduct			Right Oviduct				
	Left Ovary	Right Ovary	Max. mm Hg	Min. mm Hg	mm Hg3	Max. mm Hg	Min. mm Hg	mm Hg3		
1	-	F	250	190	94	140	100	80	+	180
2	f CL	-	230	164	80	210	120	86	+	100
3	CL	F	100	57	40	94	41	32	-	-
4	ff	f -	110	66	45	95	55	50	-	-
5	FF CL	-	170	74	64	140	86	27	+	140
6	f	-	80	33	22	165	90	82	+	145
7	-	-	165	76	72	190	60	43	+	160
8	f	f (CL)	90	42	30	110	76	72	-	-
9	-	f	170	43	43	235	124	46	+	190
10	-	F CL	110	76	52	208	93	45	+	130
11	-	CL	150	85	38	180	118	58	+	80
12	f	(CL)	170	106	56	177	126	40	+	120
13	-	-	252	123	93	140	66	59	-	-
14	-	F	240	64	47	138	94	62	-	-
15	F	-	160	130	50	145	78	62	-	-
MEAN			163.13	88.60	55.07	157.80	88.47	56.27		137.22
STANDARD DEVIATION			58.58	45.64	21.60	42.22	26.30	18.10		35.10
STANDARD ERROR			15.12	11.79	5.58	10.90	6.79	4.68		11.70

KEY: CL = Corpus luteum over 2 cm in diameter: F = Follicle over 1.5 cm in diameter: f = Follicle over 1 cm and under 1.5 cm in diameter.
(CL)= Growing corpus luteum

All this was done in automatic tissue processing machine (Shandon Elliot). The specimens were then blocked out in paraffin wax and sections were cut at 5U. The sections were mounted on slides in Harlecos synthetic resin and stained with Mayers hamalum and eosin. The slides were left to dry for 24 hours prior to examination.

Results

Table 3 shows the results of the maximum and minimum readings of air pressures recorded when passing air through the oviducts from uterine end towards the ovary in 15 bovine genital tracts in vitro.

A total of 85 genital tracts were examined. The results of 15 animals recorded in Table 3 are a representative sample of the results obtained.

No significant difference between the pressures required to pass air through the left and the right oviducts was recorded. In addition there was no statistical difference in the pressure required in the presence of either corpora lutea or follicles on the ovary.

Note that endometrial rupture occurred in 7 of the 15 recorded cases with a wide variation at where the rupture occurred.

There was no significance in the pressures recorded between the left and right oviducts ($p = > 0.8$).

Table 4 shows results of the maximum, minimum and the third pressure recorded after removing the mesotubarium superius when passing air through the oviducts from the uterine end towards the ovary in bovine genital tracts in vitro. A total of 53 genital tracts were examined. The results of 15 genital tracts

TABLE 5

COMPARISONS BETWEEN MAXIMUM AND MINIMUM READINGS OF AIR PRESSURES RECORDED WHEN PASSING AIR UP AND THEN DOWN THROUGH THE OVIDUCTS IN BOVINE GENITAL TRACTS IN VITRO

Specimen No.	Ovarian Findings		Oviducts - Registered pressures in mm Hg							
			UP				DOWN			
	Left Ovary	Right Ovary	Left Oviduct		Right Oviduct		Left Oviduct		Right Oviduct	
			Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1	-	-	80	26	68	27	144	78	132	64
2	-	-	208	56	66	38	182	39	122	26
3	f CL	-	162	56	54	20	90	47	42	22
4	-	f CL	106	34	154	43	166	80	146	44
5	-	f	104	34	108	26	200	80	110	44
6	-	-	160	38	66	38	230	10	220	32
7	-	-	158	13	118	38	88	8	108	36
8	-	f	102	32	162	22	100	40	180	19
9	-	-	70	24	118	40	152	64	169	36
10	-	-	110	50	58	26	94	55	84	26
11	-	-	108	78	70	35	230	90	146	54
12	-	-	78	20	81	31	122	56	126	48
13	-	-	82	47	162	47	106	42	100	70
14	-	-	102	55	178	96	240	110	180	68
15	-	-	70	39	130	80	110	58	126	57
16	-	f (CL)	116	69	108	42	184	90	145	31
17	-	-	162	36	64	40	106	44	70	42
18	-	-	146	34	86	24	114	66	118	70
19	-	-	70	36	116	46	90	42	114	66
20	-	-	80	48	Blocked		200	25	Blocked	
MEAN			113.70	41.25	103.53	39.95	147.40	56.20	128.32	45.00
STANDARD DEVIATION			39.38	16.21	39.69	18.99	52.55	26.73	41.53	17.13
STANDARD ERROR			8.81	3.63	9.11	4.36	11.75	5.98	9.53	3.93

t-test between pressures up and down

KEY: CL = Corpus luteum over 2 cm in diameter: F = Follicle over 1.5 cm in diameter: f = Follicle over 1 cm and under 1.5 cm in diameter
(CL) = Growing corpus luteum.

recorded in Table 4 are a representative sample of the results obtained. Note that endometrial rupture occurred in 9 of the 15 genital tracts used with a wide variation at site of rupture.

The fall in pressure from minimum to the third pressure was statistically significant on T test ($p = < 0.001$), but there were no statistically significant differences in the pressures recorded to pass air through the oviducts between the left and the right oviducts ($p = > 0.7$), between genital tracts with or without corpora lutea ($p > 0.9$) or follicles over 1.5 cm diameter ($p = > 0.8$) on the ovary.

Table 5 shows the results of the maximum and minimum readings of air pressures recorded when passing air up and then down the oviducts in bovine genital tracts in vitro. The pressure required to pass air down the oviduct was greater and this was shown to be statistically significant ($p = < 0.01$) on T test.

Discussion

One of the technical difficulties experienced when carrying out the air insufflation test on bovine genital tracts was leakage of air from the cervix. The modified catheter described by Hanley (1953) was not available but even the use of a Foley's catheter did not overcome the problem completely. Although in the living animal the cervix is described as a firm tightly closed structure it becomes slack and dilated at oestrus, during the post-partum period before uterine involution has occurred and in some pluriparous cows, at all stages of the oestrous cycle.

Therefore in considering the use of this test as a diagnostic aid for patency of the oviducts in cattle, the possibility of air escaping from a dilated cervix should be borne in mind.

However, the major disadvantage of the use of this test in cattle is that it can cause rupture of the endometrium. Rupture of the endometrium following the use of the air insufflation technique has been reported by Hanley(1953) and Kawata and Koike (1959). In the study reported in this thesis rupture of the endometrium occurred in 31% of the post-mortem specimens in which the test was carried out. Rowson (1942), Hanley (1953) and Becse and Stark (1968) reported similar findings. In the experiments using the insufflation method described in this thesis, rupture of the endometrium always occurred at the lesser curvature of the uterine horns and the air escaped into the broad ligament, accumulating between its double peritoneal layers. Hanley (1953) and Kawata et al. (1959) reported the same findings in genital tracts removed from cows slaughtered immediately after carrying out this technique. It would appear that the endometrium ruptured when the elastic

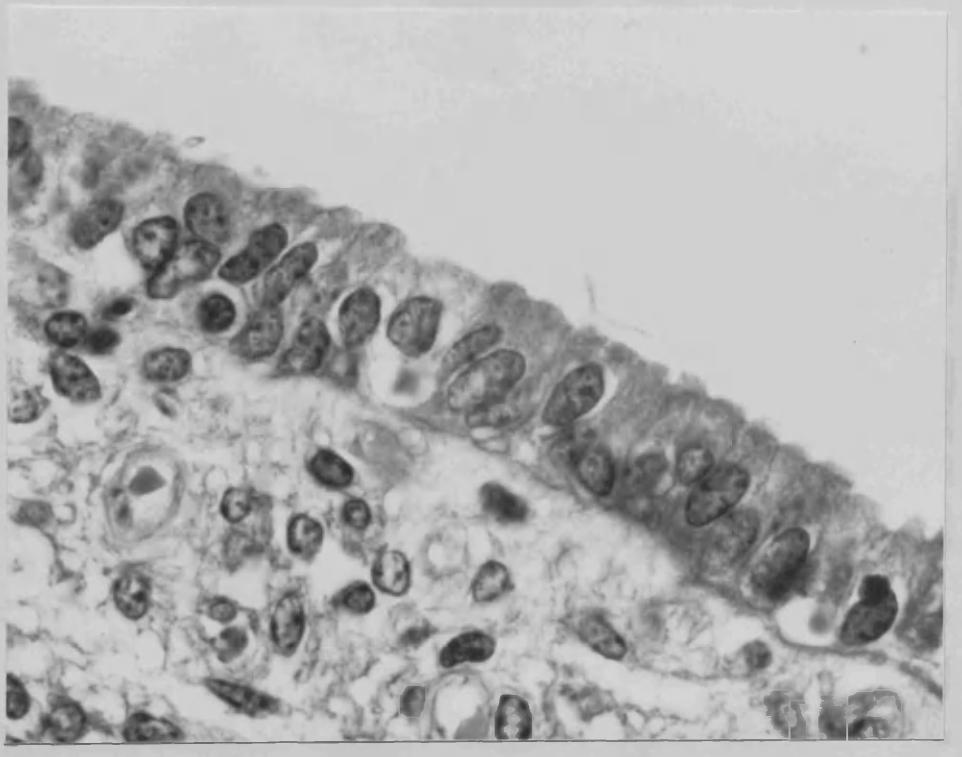
limit of the uterus was passed and there was no other outlet for the air at this pressure. The fact that the rupture always occurred at the same place suggests that this was the area of least resistance and could be explained by the lack of serosa at this site. However, rupture of the endometrium was not always associated with obstruction of the oviducts. Indeed in 98% of cases where endometrial rupture occurred both the oviducts were patent and in the remaining 2%, one of the oviducts was patent. Furthermore there was no significant difference in the pressure required to demonstrate patency in the oviducts in those cases where rupture occurred and in those where it did not. This could suggest that in those genital tracts in which rupture occurred the endometrium was more friable. However, in the study described in this thesis only uteri that were apparently normal on gross examination were used. Furthermore, subsequent histological examination of sections taken from uteri where rupture occurred when compared with sections in which rupture did not occur revealed no histological differences. Comparison between the two types of endometrial specimens showed no leucocytic infiltration or damaged or detached epithelial cells in the sections where rupture had occurred. However, although such histological examination would have indicated obvious differences in the structure of the endometrium in cases where rupture had or had not occurred, it would be of interest to examine specimens from the endometrium where rupture had occurred and compare these with specimens where rupture had not occurred using electronmicroscopy to see if at this much greater magnification differences in cellular structure might have been demonstrated.

In cattle during pro-oestrus and on days 8 and 10 of the

oestrous cycle there is an increased vascularisation and oedema of the uterus and during metoestrus there is some disruption of the caruncular epithelium. These changes might explain why rupturing of the endometrium occurred in some cows subjected to the air insufflation test. Since in the experiments carried out in this study, post-mortem specimens were used, it was not possible to apply this suggestion to our findings. However, when comparisons were drawn between those cases which had obvious corpora lutea in their ovaries and those which had not, no correlation was found between these findings and the absence or presence of uterine rupture during the carrying out of the insufflation technique. However, it would be of interest in attempting to explain the uterine rupture to determine the exact stage of the cycle by either behavioural studies or hormonal estimations and at these exact stages of the cycle to carry out the air insufflation test and after slaughter to examine the tracts for the presence of uterine rupture.

In the genital tracts examined in this study, the area of the oviducts found to be most resistant to the passage of air during the transuterine insufflation was the isthmus. The isthmus is the area of the oviduct in cattle that has the narrowest lumen and the thickest wall. It is a relatively firm undilatable structure and these features could account in part for the resistance to the passage of air. In addition the sharp flexure found at the utero-tubal junction could add considerably to the resistance to the flow of air. During insufflation this flexure was found to be most marked when the uterine horns were coiled up. Dissecting out the mesotubarium superius almost straightened out this flexure. Also, in the specimens where the oviducts were patent when the air

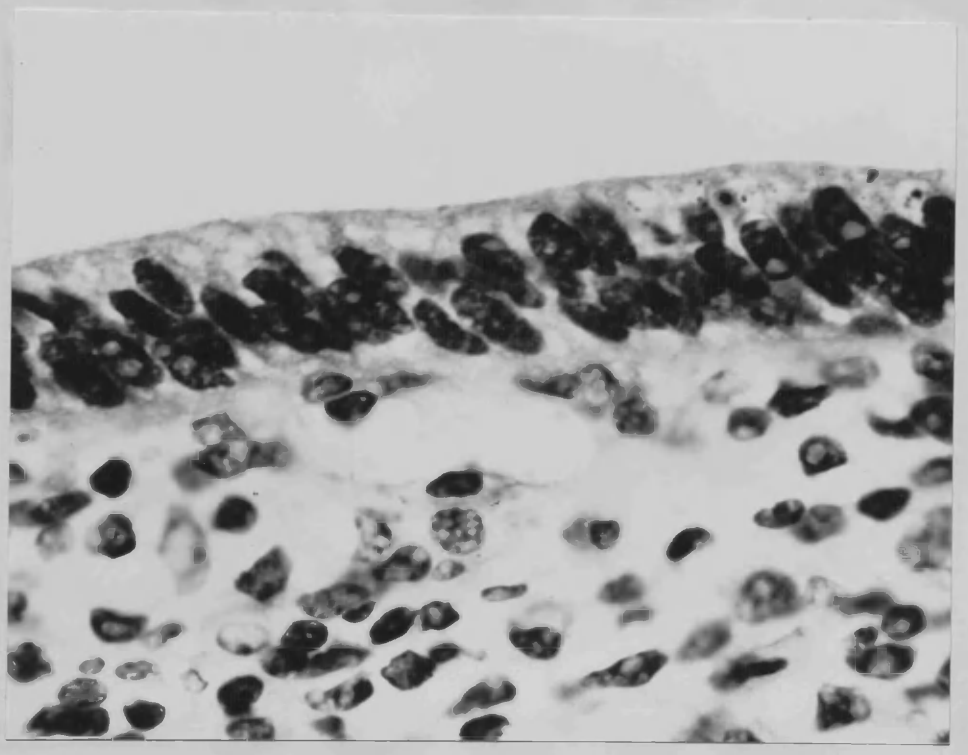
FIGURE 2



X1000 H & E

Figure 2 shows a section of endometrium from an apparently normal bovine genital tract.

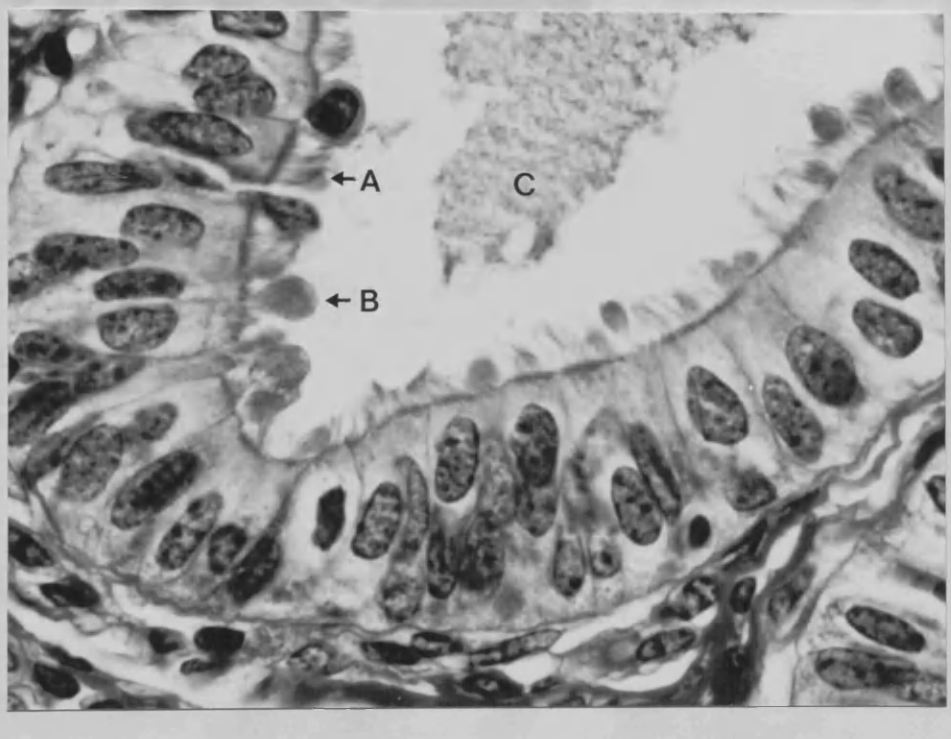
FIGURE 3



X1000 H & E

Figure 3 shows a section of endometrium from an apparently normal bovine genital tract after passing air. There is no obvious difference in comparison with the section from apparently normal endometrium.

FIGURE 4

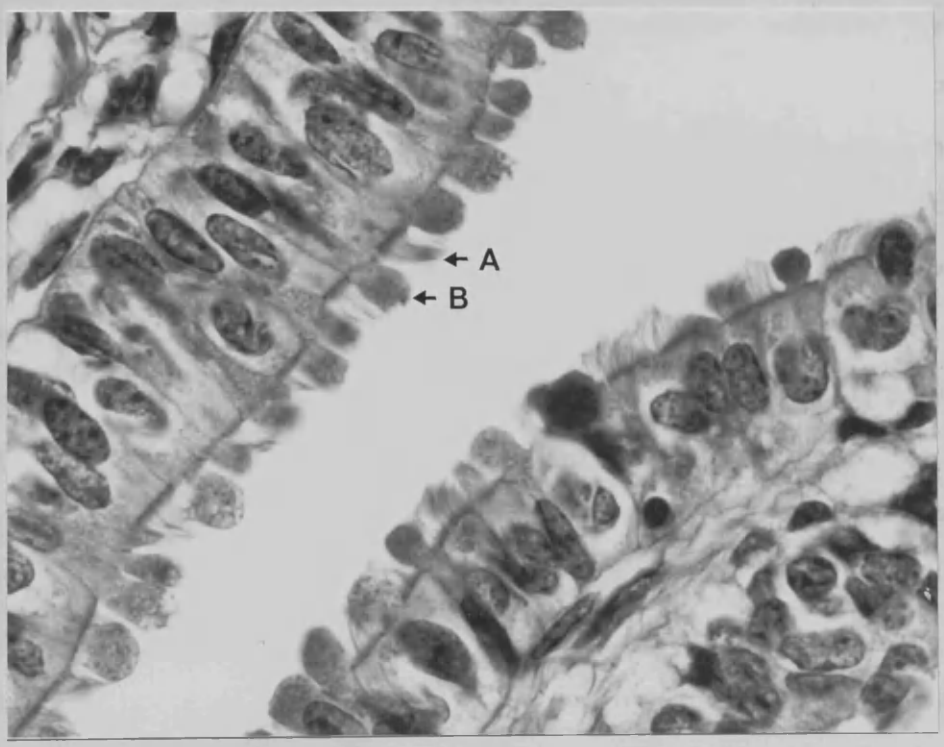


X1000 H & E

Figure 4 shows a section from the ampullary region of the oviduct from an apparently normal bovine genital tract.

Note the cilia (A) and cytoplasmic protrusions projecting from the epithelial cells (B), and debris in the lumen (C).

FIGURE 5



X1000 H & E

Figure 5 shows a section of the ampullary region of the oviduct from an apparently normal bovine genital tract after passing air.

There is no obvious loss of cilia (A), and cytoplasmic protrusions (B). Note the absence of debris in the lumen.

was passed upwards through the oviducts to escape into the peritoneal cavity the pressure fell with the escape of the gas to a steady low level. After carefully cutting the mesotubarium the pressure fell further indicating that removal of the ligament allowed more air to escape from the oviduct. Dissecting out this ligament also reduced the tortuousness of the isthmus, again accounting for the lowering of this pressure. One might suggest that in the living animal around oestrus the elevated levels of oestrogen in the circulating plasma could cause slackening of this ligament as occurs with other ligaments associated with the reproductive tract, e.g. the pelvic ligament. At oestrus, however, the uterus is known to be turgid and tightly coiled resulting most probably in a tightening of this ligament and an increase in the flexure of the isthmus. However, since ovulation in the cow does not occur until 13-16 hours after the end of oestrus, the uterus has by then lost most of its turgidity and the slackening of this ligament might well play a part in the descent of the fertilised ova down the oviduct to the uterus.

Once again, however, attention must be drawn to the fact that these experiments were carried out on post-mortem specimens and an extrapolation of results recorded under these circumstances to the living animal should be assessed carefully. However, this does not exclude the indications of areas of investigation in the living animal from results obtained <u>in vitro</u>.

As far as could be ascertained from the subsequent examination of the sections taken from the oviducts in which the air insufflation test had been carried out <u>in vitro</u>, there were no obvious indications that damage had been inflicted to the

lining of the oviduct in the carrying out of this procedure. There was no increase in the number of detached cells. The cilia of the cells lining the lumen of the oviducts remained intact but there was an obvious emptying of the oviduct after the application of the test as compared with the sections from those specimens where no test had been carried out. In the untreated cases the lumen of the oviduct was seen to be occupied by detached cells and numerous cell protrusions known to be numerous in certain regions of the oviducts. As well as this cellular debris there was oviductal secretion which stained an obvious faint pink in the sections examined from the untreated specimens. It was obvious that the air insufflation technique had caused a flushing out of the oviducts.

Kowaliszyn (1969) reported that after carrying out the insufflation test cows that had previously been infertile demonstrated an improvement in fertility. However, he somewhat complicated the claim for simple air insufflation by administering cortisone and novocaine to the cows. The air insufflation technique in the human is one that is frequently advocated and it would seem that from this point of view the air insufflation technique might have similar advantages in cattle.

One might have expected that the pressure required to pass air down the tube after initially passing it up the tube would be less because any obstruction within the lumen of the oviduct would have been removed by the initial passage of air. This, however, was found not to be the case, ~~more~~ greater pressure being required to pass air down the tube and the difference between the two different approaches was significant.

This has already been reported in the literature by Andersen in 1928 in which she carried out this study in a very small number of genital tracts of cattle. There could be two different explanations for this, firstly, it seems reasonable when one considers the oviducts as a tube with a lower firm segment having a narrow lumen and an upper distendable segment with a wider lumen that the passage of air from the narrow segment towards the open end of the wide segment would require less pressure than in the reverse situation. This could explain the greater pressure required to pass air down the oviduct. In addition the fact that air was initially passed up the oviduct would cause the mucosal folds lining the lumen to be directed towards the open ovarian end of the oviduct. These folds would then act as a valve during the subsequent passage of the air down the oviduct. This valve-like effect would be more pronounced where these folds were well defined and complex in the arrangement, viz. in the infundibulum and the ampulla. Since very low pressure caused ballooning of the ovarian end of the oviduct this valve-like effect was minimal in this segment. However, these folds, projecting into the lumen of the ampulla may be effective in causing an obstruction by impeding the flow of air at the ampullary isthmus junction where there is a narrowing of the tube as well. In addition the ballooning of the ampulla would cause the isthmus at the AIJ to be pulled inwards and to project into the lumen of the ampulla causing a further obstruction.

Andersen (1928) passed saline under pressure through fresh specimens of cattle oviducts from areas around the UTJ and reported that it required more pressure to pass fluid from the

oviduct end than from the uterine end. This she reported as a consistent finding in all four tracts she examined, one of them had just ovulation, two had large follicles and one had a regressing corpus luteum.

Black and Davis (1962) passing air down the oviduct demonstrated that there was resistance created by the isthmus. They, however, found that the removal of the UTJ did not markedly reduce the amount of pressure required to pass air through the oviduct. They demonstrated, in cattle, that for 72 hours after ovulation the flow of oviductal secretion is towards the ovarian end. Furthermore if during the 72 hours the ovarian end is tied off, the secretion collected within the oviduct causing distention of the ampulla did not flow downwards into the uterus immediately. From 72 hours after ovulation this fluid gradually escapes via the isthmus. These findings suggest a partial temporary blockage within the oviduct to passage of any fluid in a downward direction. It is during this period that inseminated spermatozoa travel through the uterus up to the oviduct to the site of fertilisation, i.e. the ampulla.

Although as stated previously the work described in this thesis was in post-mortem specimens where such findings cannot be applied, it is of interest to consider them in regard to discussing the difference in pressures found during the insufflation technique and to again indicate the areas for further investigation.

In conclusion, considering the problem of leakage of air, the high incidence of endometrial rupture, the risk of introducing infection into the uterus, the insufflation test of the oviducts would not appear to be a suitable technique for the diagnosis of oviduct patency in cattle.

SECTION 2

PHENOLSULPHONPHTHALEIN (P.S.P.) DYE TEST

PHENOLSULPHONPHTHALEIN (P.S.P.) DYE TEST

Introduction

The results of the P.S.P. test in cattle already reported in the literature are, on the whole, encouraging. However, the use of the test in cattle still requires investigation. Little is known about the effect the different stages of the oestrous cycle have on the results of such a test, the subsequent fertility of cattle in which the test has been carried out and whether or not the P.S.P. test gives a clear indication of the presence of normal, unilateral or bilateral occlusion of the oviducts in cattle. It was therefore decided to carry out the dye test in cattle in an attempt to gain information about this test in these indicated circumstances.

Materials

Animals - Cows and mature heifers of various breeds, sizes and ages were used.

Preparation of animals

The animals selected for the dye test were first examined rectally to determine the size of the uterus and ovaries and to note ovarian structures. Behavioural studies were carried out so that animals in standing oestrus were recorded.

The animal was restrained in a cattle crush and the tail was tied to the halter for ease of working and to avoid soiling of the perineum and the external genitalia. The perineum and vulva were thoroughly cleaned with soap and water. This was repeated at all times during the dye test when soiling of the area occurred. The entire procedure was carried out maintaining maximum obstetrical hygiene to avoid infection as many tests were done in animals in metoestrus and dioestrus.

The equipment was sterilised prior to use.

Neilson's metal catheter.

Plastic pipettes.

0.03% alkaline solution of P.S.P. dye.

1 N sodium hydroxide.

20 ml universal bottles.

20 ml syringes.

P.S.P. dye solution

The P.S.P. dye solution was prepared by dissolving 0.3 gms of phenol red of pH range 6.8-8.4 and 4.2005 gms of sodium bicarbonate anhydrous in 1000 ml of deionised water. The resulting mixture was shaken thoroughly to dissolve the components. It was then filtered

through Millipore using 0.45μ pore size filter.

The filtered dye was put in previously sterilised 20 ml universal bottles and resterilised at 15 lbs/square inch pressure at 121°C for 15 minutes in a portable steam steriliser.

Preparation of 1 Normal sodium hydroxide

This was done by dissolving 40 gms of sodium hydroxide in 1000 ml of deionised water.

Neilson's catheters

These were placed in individual polythene bags and sealed with temperature indicator tape. They were sterilised in a vertical steam steriliser (Thackeray type).

Plastic pipettes

These were packed individually as the Neilson's catheter and sterilised in ethylene oxide (4) at 55°C for 1 hour in the Victoria ethylene oxide steriliser Mark II.

Method

The cervix was grasped per rectum and a sterile Neilson's catheter was then introduced into the vagina, through the cervix and into the uterus. Once the catheter was in the body of the uterus it could easily be felt per rectum through the uterine wall.

The dye was introduced via the catheter into the uterus still holding the catheter in position per rectum until distention of the uterus occurred. The amount of dye required depends upon the size of the uterus. Ten ml of air was injected into the catheter to ensure complete instillation of the dye and the catheter was removed with the syringe attached to it to avoid any contamination of the vagina.

Collection of urine samples

An initial urine sample was collected by passing a plastic pipette via the urethra into the bladder and the bladder was then emptied. Urine samples were collected at 5, 15, 30, 45, 60, 90 and 120 minutes after injection of the dye. To all urine samples a few drops of 1 N sodium hydroxide were added to render the urine alkaline so that the colour reaction was demonstrated. After getting 2 positive urine samples, further urine collection was not carried out except at 120 minutes after injecting the dye.

The depth of the colour of the dye in urine was assessed visually.

Post-operative treatment

Terramycin Q 50 mg/ml was administered intravenously for 3 days after the dye test, the volume depending upon the size of the animal.

LIGATION OF OVIDUCTS

Preparation of animals

One cow and one maiden heifer were used in this experiment. The animals were starved for 24 hours and water was withheld for 12 hours prior to surgery. The animals were restrained in a tilted cattle crush and the rectum emptied prior to commencing the operation to allow more room in the abdominal and pelvic cavities for manipulation of the genital tract. A left flank approach was adopted.

The operation site was clipped, shaved and cleaned with warm water and cetavlon. These areas were dried and povidone iodine applied.

Anaesthesia

The thirteenth thoracic and the first three lumbar spinal nerves were blocked by paravertebral anaesthesia as described for endoscopy.

Method

Approximately 7" long skin incision was made in the left flank. The external abdominal oblique muscle was incised in the direction of the skin incision. The external fascia of the internal oblique was incised. The internal fascia of the internal abdominal oblique muscle and the peritoneum were cut in the direction of the muscle fibres of the internal abdominal oblique muscle and air was heard rushing in creating a pneumoperitoneum.

A hand was introduced via the incision and the genital tract was located. The left oviduct was located by following the left uterine horn from the bifurcation towards the tip and near the tip of the uterine horn the oviduct was felt as a firm cord-like

structure. The other hand was introduced with a half-circle round-bodied needle to which a long No. 1 linen suture was attached. The needle was inserted in the mesosalpinx to include the isthmus and this was tied off with a surgeon's knot. Another ligature was placed in a similar manner near the ovarian end of the oviduct.

Suturing technique

The peritoneum was closed with No. 8 chromic catgut in a simple continuous pattern. The internal and external oblique muscles were sutured separately in the same way, care being taken to include the internal and external fascia of the muscle. The subcutaneous tissue was sutured with chromic catgut No. 7 in a simple continuous suture pattern to avoid formation of pockets of serum or blood under the skin. Skin was sutured with No. 2 nylon with interrupted horizontal mattres suture pattern. The wound was sprayed with terramycin aerosol spray.

Post-operative treatment

Terramycin Q 50 mg/ml was administered intravenously for 4 days, the dose depending upon the size of the animal. The skin sutures were removed on the 7th post-operative day.

The right oviduct was ligated similarly from the right flank in the cow after 40 days.

HISTOLOGY

Materials and methods

Seven genital tracts of animals previously subjected to the P.S.P. dye test were collected immediately after slaughter 2 hours to 7 days after the last test. In 5 of these oviduct patency was checked by transuterine insufflation with air.

The genital tract findings on gross examination were recorded. All of the uteri were cut open and any abnormalities noted. A 2 cm square piece of both uterine horns was taken just anterior to the external bifurcation of the uterine horns.

Both oviducts were dissected free from their attachments and 1 cm of the fimbriated portion of the oviduct, 1 cm piece of the ampulla, 5 cms from the ovarian open end of the oviduct and 1 cm piece of isthmus 5 cms away from the UTJ were collected from both oviducts.

Preparation

All specimens were collected and fixed within 15 minutes after slaughter and were prepared as described in the previous section.

Results

Table 6 shows the results of the P.S.P. dye test in a random sample of 10 cows in which 20 ml of the P.S.P. dye solution was instilled into the uterus and the initial appearance of the dye in urine recorded. Note that in 2 cows in which dye was not recorded in the urine, bilateral patency of oviducts was demonstrated on examination of genital tracts immediately after slaughter. The 2 cows in which dye was initially detected in the urine at 60 minutes had unilateral occlusion of oviducts.

Also note the variation in the time when the dye was first detected in the urine in 5 cows which were shown to have bilateral patency of oviducts on examination of genital tracts after slaughter.

Table 7 shows the results of the dye test in 3 cows when different amounts of the dye were introduced into the uterus. Note that in 2 cows in which on first carrying out the dye test no dye was found in the urine within 2 hours. Subsequently, after increasing the volume of the dye, dye was found in the urine within 30 minutes and both oviducts were shown to be patent on examination of the genital tract immediately after slaughter.

Table 8 shows the results of the P.S.P. dye test in 6 cows repeated at various stages of the oestrous cycle. No obvious differences in the time of first appearance of the dye in urine are apparent.

Note the reduction in time recorded for the initial appearance of the dye in the urine on the second test in Cow 26 and on the third test in Cows 23 and 26. Also note that there is an increase in the time of initial appearance of the dye in urine in

TABLE 6

RESULTS OF DYE TEST IN A RANDOM SAMPLE OF COWS

Cow No.	Amount of Dye ml	Stage of Cycle	Time (mins) first appearance of dye in urine	Post-mortem findings
1	20	Dioestrus	-	Normal
2	20	Metoestrus	-	Normal
3	20	Ovariectomised	15	Not slaughtered
4	20	Dioestrus	15	Normal
5	20	Oestrus	30	Normal
6	20	Anoestrus	60	Right oviduct blocked
7	20	Anoestrus	30	Normal
8	20	Cystic ovary	30	Normal
9	20	Anoestrus	45	Normal
10	20	Dioestrus	60	Left oviduct blocked

TABLE 7

RESULTS OF DYE TESTS IN 3 COWS WHEN DIFFERENT AMOUNTS OF DYE WERE INTRODUCED INTO THE UTERUS

Cow No.	Time of first appearance of the dye in urine		Stage of Cycle
	20 ml dye	40 ml dye	
11	-ve up to 2 hrs	30 mins	Metoestrus
12	-ve up to 2 hrs	30 mins	Metoestrus
13	-ve up to 2 hrs	-ve up to 2 hrs	Anoestrus*

* P.M. findings - both oviducts blocked.

TABLE 8

RESULTS OF DYE TESTS IN COWS REPEATED AT VARIOUS STAGES OF OESTROUS CYCLE

Cow No.	Amount of Dye (ml)	Time (mins) of first appearance of dye in urine					
		Oestrus		Metoestrus		Dioestrus	
21	30	15	(II)	30	(III)	15	(I)
22	40	15	(II)	15	(III)	15	(I)
23	40	45	(I)	45	(II)	15	(III)
24	20	15	(I)	15	(II)	15	(III)
25	20	30	(I)	Not done		30	(II)
26	20	30	(II)	15	(III)	45	(I)
Average time		25		24		22.5	

(I) = First dye test

(II) = Second dye test

(III) = Third dye test

P.M. Findings: Cow Nos. 1, 3 and 5 - Both oviducts patent.

Cows Nos. 2 and 6 conceived to first insemination and are pregnant.

TABLE 9

RESULTS OF THE DYE TEST WHEN REPEATED IN THE SAME COW AT DAILY INTERVALS FOR A TOTAL OF 3 DAYS AND THEN AT 3 DAY INTERVALS ON THREE OCCASIONS

Cow No.	Amount of Dye ml.	Time of 1st appearance of dye in urine on days (mins)						Reproductive state of the cow
		1	2	3	6	9	12	
31	20	15	15	15	15	15	15	Cycling
32	60	15	15	15	15	15	15	Cystic ovary
33	30	30	15-30	15	15	15	15	Anoestrus
34	40	45	30	15	15	15	15	Anoestrus
35	20	30	15	15	15	15	15	Anoestrus
36	40	30-45	30-45	15	15	15	15	Cycling

TABLE 10

RESULTS OF DYE TEST IN COWS IN WHICH OVIDUCTS WERE LIGATED

Cow No.	Amount of Dye ml.	Time (mins) of first appearance of the dye in urine		
		Normal	Ligation	
			1 Oviduct	2 Oviducts
41	20	30	60	*
42	20	15	60	90-120

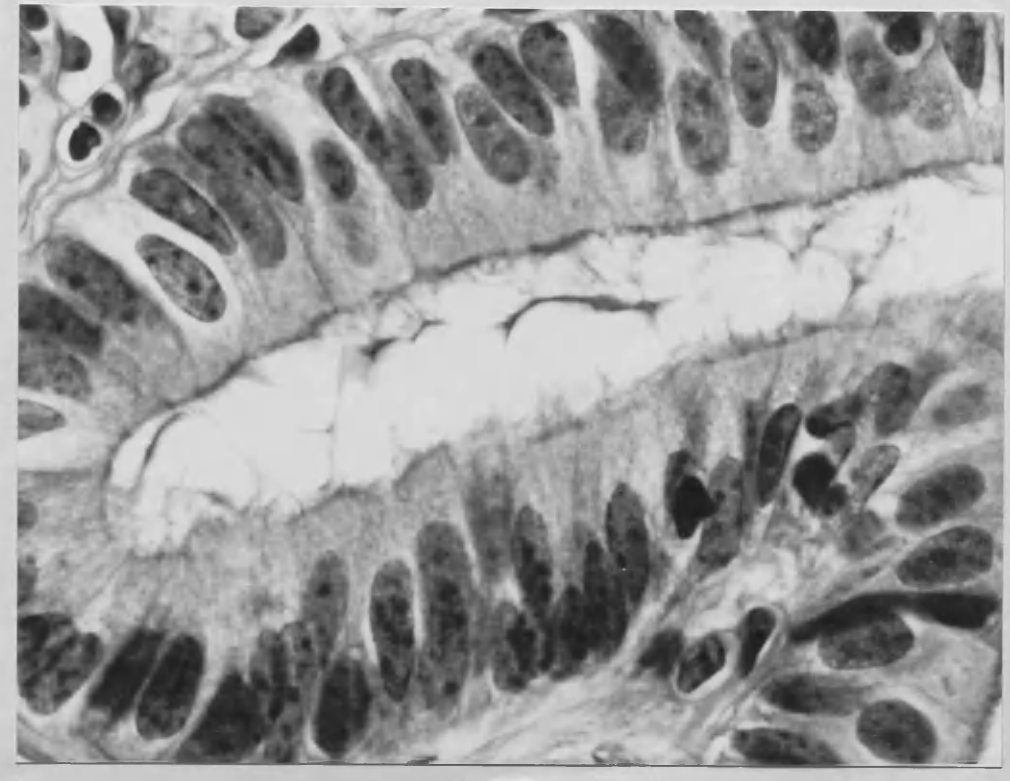
* Not ligated.

TABLE 11

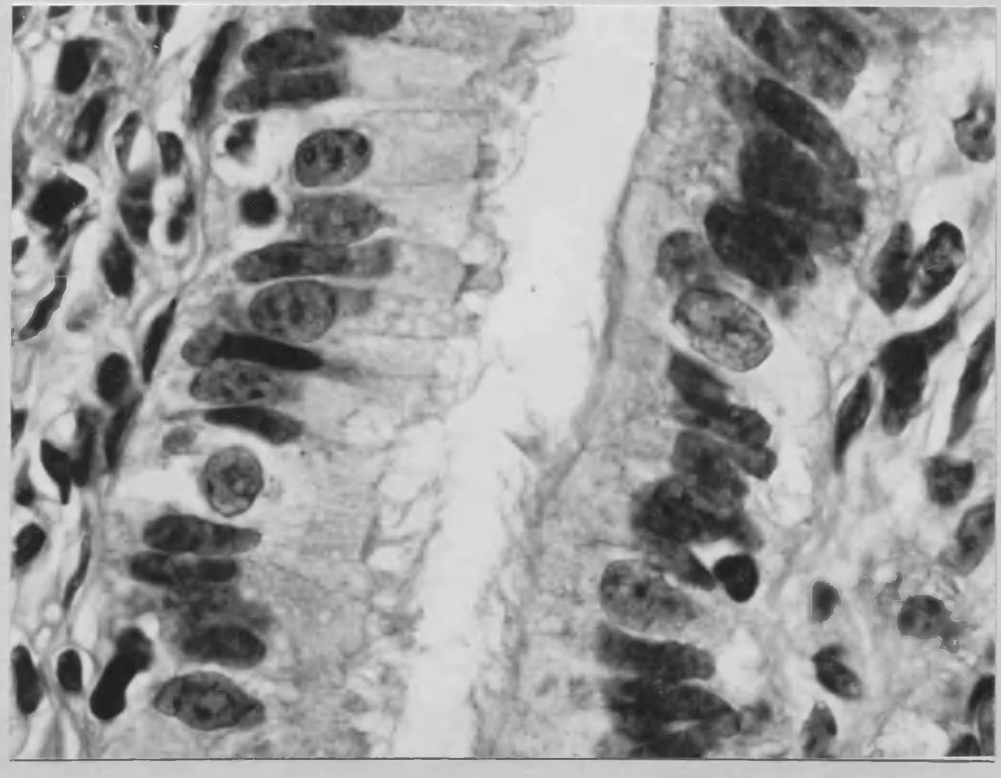
SUBSEQUENT FERTILITY IN CATTLE IN WHICH THE P.S.P. DYE TEST HAD BEEN CARRIED OUT

Cow No.	Breed	No. of Dye Tests	No. of Services	State
51	Red Poll	4	1	Pregnant
52	Dexter	2	1	Not pregnant
53	Ayrshire	4	1	Pregnant
54	Friesian	5	1	Pregnant
55	Friesian	5	1	Pregnant

FIGURE 6



A



B

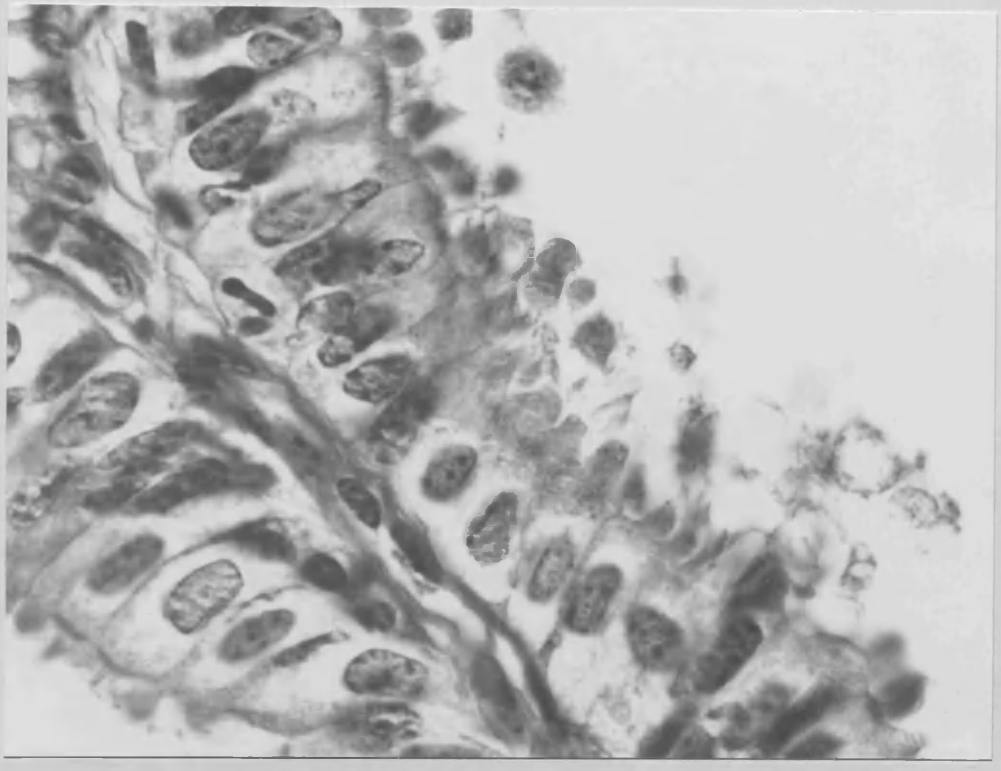
X1000 H & E

Figure 6 shows a section of the isthmus from

A an apparently normal oviduct

B an oviduct after carrying out the dye test.

FIGURE 7



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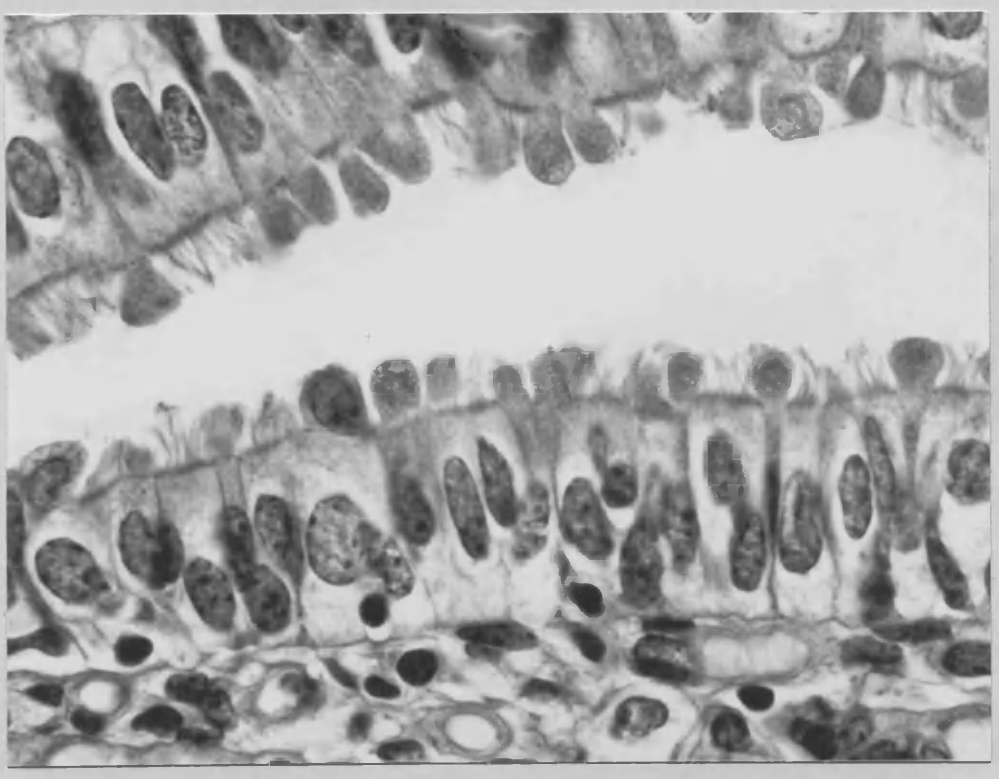


B

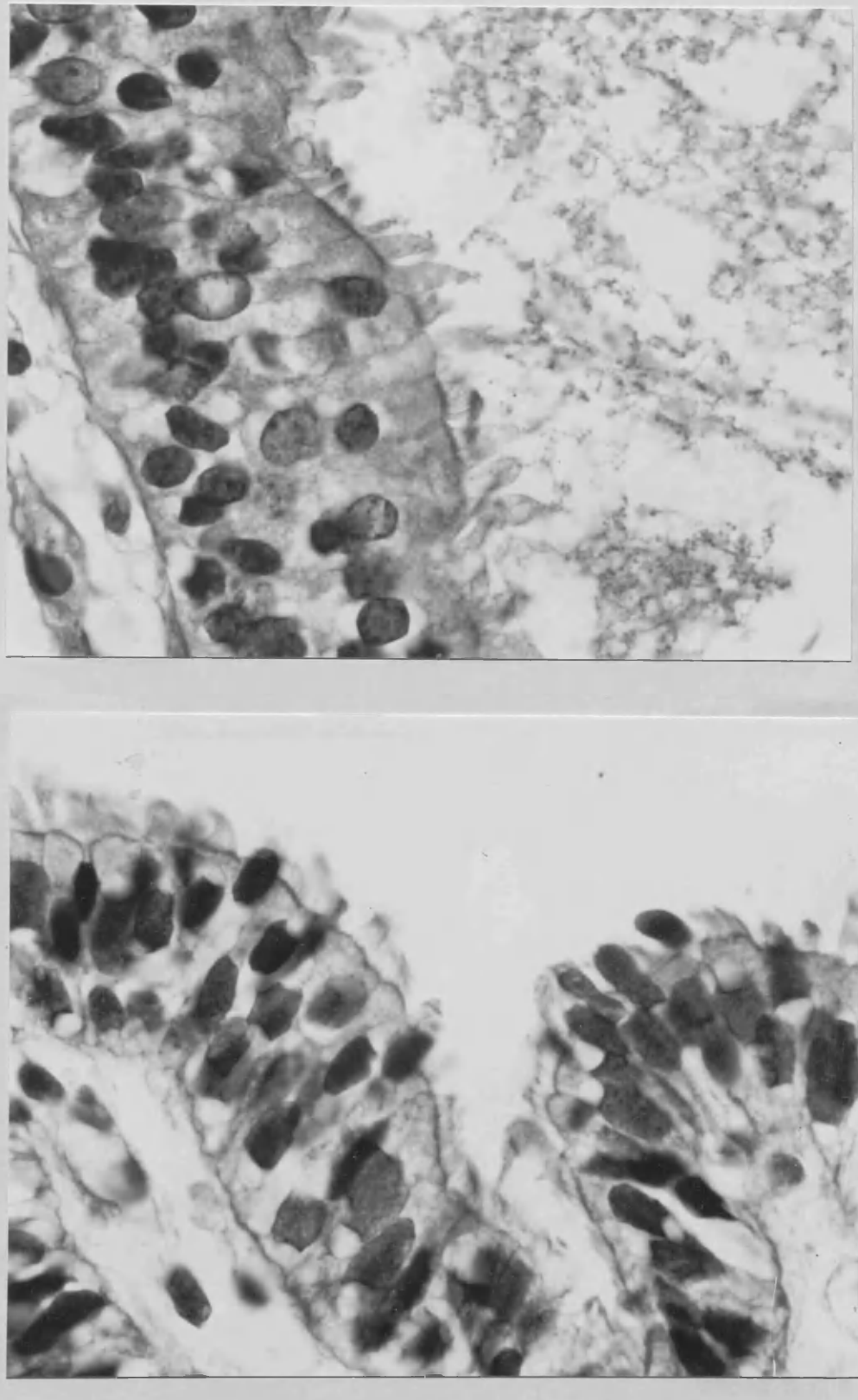
X1000 H & E

Figure 7 shows a section of the ampulla from

A an apparently normal oviduct

B an oviduct after carrying out the dye test.

FIGURE 8



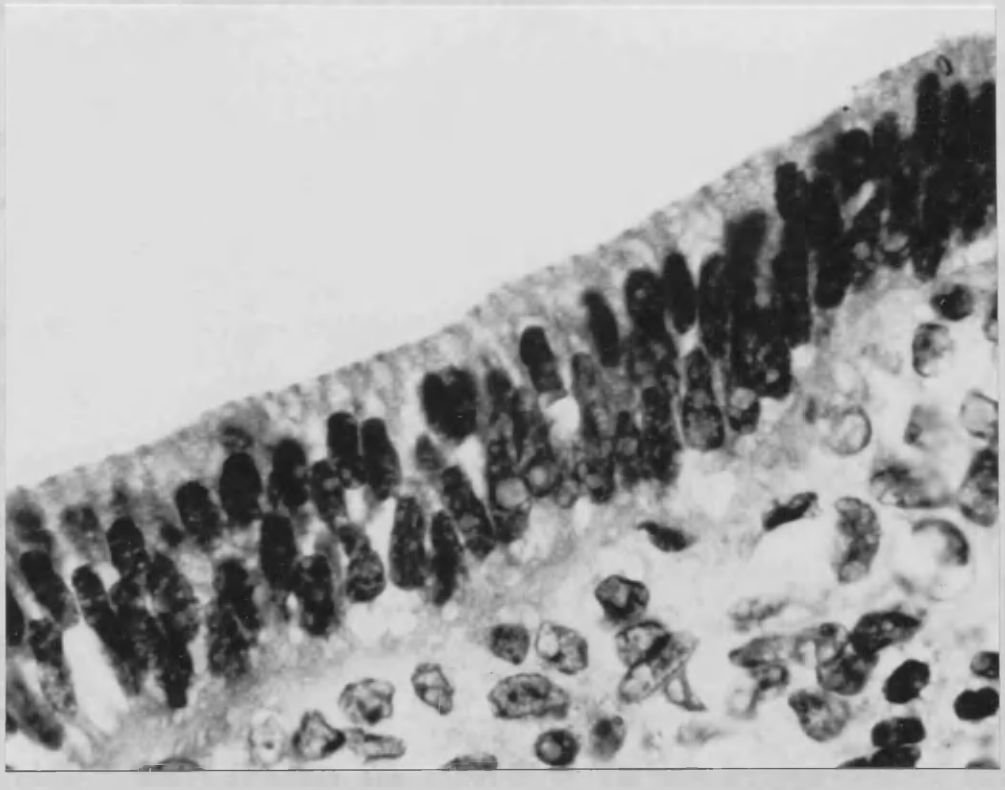
X1000 H & E

Figure 8 shows a section of the infundibulum from

A an apparently normal oviduct

B an oviduct after carrying out the dye test.

FIGURE 9



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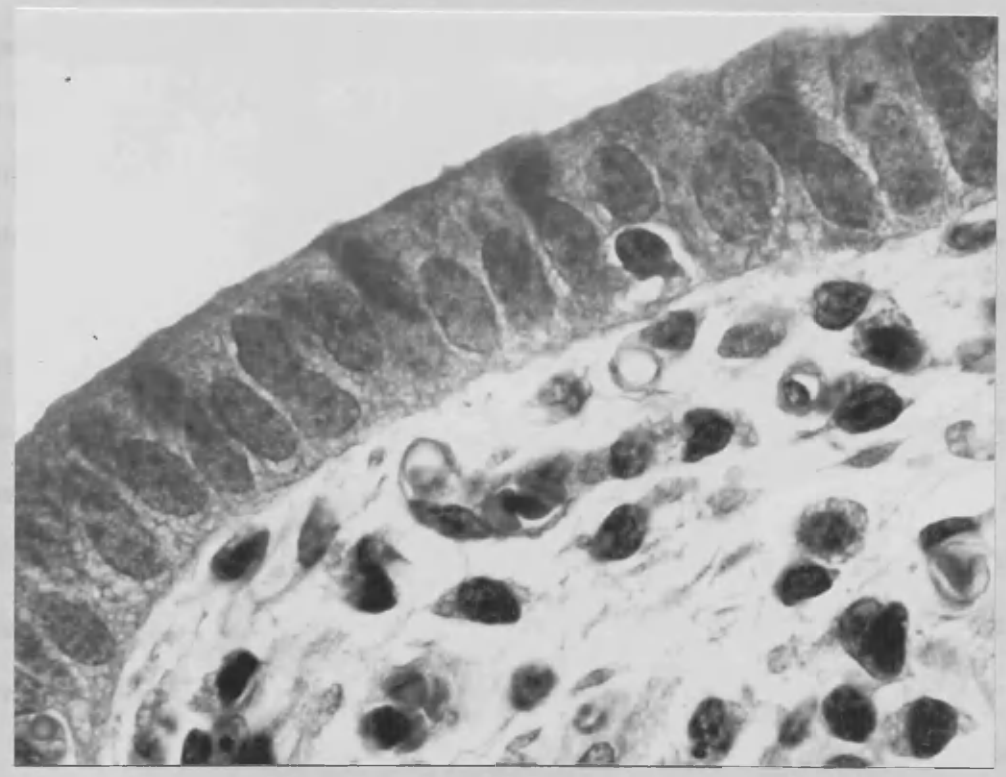


B

X1000 H & E

Figure 9 shows a section of the endometrium from

A an apparently normal genital tract

B a genital tract after carrying out the dye test.

Cow 1 on the third test in metoestrus.

Table 9 shows the results of the dye test when repeated at daily intervals for a total of 3 days and then at 3-day intervals on 3 occasions.

Note the reduction in time of initial appearance of the dye in urine recorded in the second test (Cows 33, 34 and 35) and in the third test (Cows 33, 34 and 36). Also note that in these cows 15 minutes, the minimum time recorded on the third test, was also recorded at the subsequent tests.

Examination of the genital tracts of 4 cows (32, 33, 34 and 35) slaughtered subsequently demonstrated bilateral patency of oviducts in all 4 cows.

Table 10 shows the results of the P.S.P. dye test in animals in which oviducts were ligated. Note the increase in time of initial appearance of the dye in the urine after ligating one oviduct in the animals, and the further delay in the initial appearance of the dye in the urine in Cow 42 after ligating the second oviduct.

Table 11 shows the subsequent fertility in cattle in which the P.S.P. dye test had been carried out. Note that 4 animals conceived to the first insemination, 3 of which are now pregnant and Cow 54 was slaughtered at 2½ months of gestation.

Figures 6, 7 and 8 show areas of the oviduct from apparently normal cattle and similar areas in cattle after carrying out the dye test. There are no obvious differences.

Figure 9 shows sections from the endometrium of an apparently normal uterus of a cow and also shows a similar section of endometrium after the dye test was carried out. Again there are no apparent differences between these two sections.

Discussion

Initial attempts at carrying out the dye test in cows very soon indicated that the passage of the catheter through the cervix, in metoestrus, dioestrus and anoestrus was not easily achieved. However, by trial and error an approach was finally developed whereby a Neilson's catheter was introduced, per vagina, as far as possible into the cervix, then by gripping the cervix per rectum the catheter was worked through the cervix until it was felt to be lying in the body of the uterus. At oestrus, on the other hand, no great difficulty was experienced in introducing the catheter into the uterus since the cervix was dilated, but the problem in these cases was the escape of the dye back into the vagina via the dilated cervix. This tended to occur in all animals where the cervix was partially dilated, for example, in cases of cystic ovaries and in some pluriparous cows. To stop the dye from escaping under these circumstances swabs were placed around the cervix immediately after removal of the catheter after instilling the dye. In addition, in all the dye tests recorded in this study the possible contamination of urine with dye was overcome by collecting urine 5 minutes after injection of the dye into the uterus. If this urine sample was found to be tinged at all with dye then the whole procedure was abandoned and the test repeated at some future occasion.

Several methods for collecting the many urine samples necessary were tried. The use on an indwelling catheter was considered. However, during several attempts at using this approach, it was found that the catheter came adrift long before the end of the experiment and the presence of the catheter in the

bladder induced straining which was felt might cause a backflow of the dye into the vagina. Finally, it was found that by introducing a well lubricated finger into the urethral orifice and guiding the catheter down this finger into the bladder between contractions of the urethral sphincter the catheter slipped into the bladder relatively easily and urine was withdrawn without any traumatic damage being done. Emptying of the bladder was always carried out before starting the test and each time a urine sample was taken. This was usually achieved without any great difficulty. However, in a few cases it was necessary to compress the bladder either per rectum or vagina before complete evacuation was achieved. It was of importance to have complete emptying of the bladder especially in those cases where occlusion of one of the oviducts was suspected. This was because, had the test been carried out when there was urine already in the bladder, this could have resulted in the further dilution of the already much reduced secreted dye and an erroneous record made of the time that the dye was first visible in the urine.

Generally speaking, the urine in cattle is alkaline, and reacts with the P.S.P. dye to give the obvious red colour. However, some cows are found to have urine with an acid pH and under these circumstances there is no colour change even when dye is present in the urine. Therefore, when carrying out the P.S.P. dye test all the urine samples collected were checked for their alkalinity and those that were acid treated accordingly. In the cows in which the dye test was carried out only 1% were found to be secreting acid urine.

Once the various technical difficulties of the carrying

out of the test had been overcome, many tests were subsequently undertaken and interesting results obtained. From the results of the studies described here it appeared that if dye was recorded in the urine of cows between 15-30 minutes of it being introduced into the uterus, this indicated both oviducts were patent. Furthermore, when dye was injected into the peritoneal cavity dye was found in the urine within 5 minutes. This would suggest that, in the normal cow with both oviducts patent, it takes 10 minutes or less for the dye to gain entry to and transverse the oviducts and escape into the peritoneal cavity.

Three animals in which dye was detected in urine within 15 minutes were slaughtered between 1½ to 2 hours after the instillation of the dye into the uterus. Dye was present on the uterine wall and in the lumen near the tips of the uterine horns. Some dye was also present in the oviducts near the fimbriated end, on and around the fimbria. These findings tend to suggest that after overcoming the resistance to the flow of the dye, there is a continuous escape of dye via the oviducts into the peritoneal cavity.

In cows in which there was a delay in the appearance of the dye, several different reasons had to be considered. Having carried out a series of experiments on the repeatability, it appeared that after carrying out the test in the same animal on at least two occasions, in some cows a reduction of the time taken for the dye to appear in the urine was recorded. Remembering the findings of the studies carried out in the post-mortem specimens, it could be that as with the air insufflation technique the passing of the dye through the oviducts caused flushing out of debris,

blood clots, etc. with a subsequent improvement of the patency of the tubes. Therefore, when a delay in time of appearance of the dye was recorded the test was repeated on at least a further two occasions before coming to any definite conclusions about permanent occlusion. However, after repeated dye tests and a persistent delay of the appearance of the dye in the urine of between 45 and 60 minutes, then this indicated unilateral blockage. In considering why such a delay occurred in these cases the work of Mixner and Anderson (1958) is of interest. They found that dye injected intravenously is excreted via the kidney into the urine within 5 minutes. Since the bladder was emptied after each collection of urine in the experiments described here, then the time taken for sufficient identifiable dye to be found in the urine in cases of unilateral blockage build up in the oviduct cannot be explained by concentration of dye collecting in the bladder. Much more logical is the explanation that there could be a renal threshhold to the excretion of P.S.P. dye from the blood of cattle and that with only one patent oviduct the time taken for accumulation of dye to reach the necessary threshhold level is approximately 45 to 60 minutes.

In their description of the P.S.P. dye test Berchtold and Brumner (1968) suggested that 20 ml should be the volume of dye used. In the first series of tests described in this study this was the amount of dye introduced. However, in several of the cows studied no evidence of dye in the urine was found for periods of upwards of 2 hours after the instillation of the dye. Post-mortem studies of the tracts of these animals did not demonstrate blocked oviducts. On further investigation it was

found that many of the cows tested had uteri of such dimensions that on instillation of 20 ml of dye no uterine distention occurred. Furthermore, in those cases in which uterine distention occurred on instillation of 20 ml of dye, immediately afterwards uterine contractions could be felt. These contractions could well play an important role in forcing the dye forwards from the uterus into the fallopian tubes and might well be necessary to overcome the apparent barrier of the isthmus junction. It was therefore decided to increase the amount of dye in cows with larger uteri from 20 ml to 40 ml. In the majority of cows treated in this way in which negative results had been recorded from previous dye tests, positive results were obtained within 30 minutes of instilling the dye in the greater quantity. It would seem, therefore, that uterine distention leading to uterine contractions did play an important role in the successful outcome of the test.

Part of the study of the P.S.P. test involved cows at the various stages of the cycle. The results recorded in this thesis gave no clear indication that there was nay notable change in the results recorded from cows whatever their stage of the cycle. One might have expected some variation during the standing oestrous period when the increased levels of circulating plasma oestrogens might have caused slackening of the mesotubarium superius ligament, resulting in reduction of the torutousness of the lower area of the tube. This could have caused quicker entry of the dye into the fallopian tubes and so a reduction in the time taken to reach the peritoneal cavity. However, perhaps a more detailed study in association with hormone assays of the plasma levels might indicate that there are changes occurring at different stages of the cycle.

In all 5 cows in which the P.S.P. dye test indicated bilateral occlusion of the oviducts, subsequent post-mortem examination confirmed these results. In one such animal slaughtered 6 hours after instillation of the dye into the uterus, no dye was detected in the urine present in the bladder. The uterus was still distended with dye and there was no dye present in either the oviducts or the peritoneal cavity. Trans-uterine insufflation confirmed non-patency of both oviducts and the endometrium appeared normal on gross and histological examination. These findings would tend to suggest that endometrial absorption of the dye is a very slow process. Therefore clear indications from the dye test carried out and the subsequent post-mortem examinations were that where uterine distention and contractions had been felt after instillation of the dye, but no signs of dye in the urine after a period of 2 hours, then such cases had bilateral occlusion of the oviducts.

To investigate whether or not the dye test causes obvious damage to the lining of the oviducts histological examination was undertaken. The area most likely to have been damaged due to the passage of the dye during the P.S.P. test is the luminal surface of the oviduct, namely the epithelium. The nature of the damage most probably would be detachment of the epithelial cells from the underlying tissues and detachment of various structures protruding from the cells into the lumen such as cilia and cytoplasmic protrusions. Detailed histological examination, paying particular attention to the above possibilities, failed to demonstrate any obvious damage to the epithelial lining, the oviducts and uterus. In those cases where the animal was not slaughtered for several days after carrying out the dye test, no significant increase in the leucocytes

present in the oviductal wall or uterus was obvious, suggesting no infectious invasion having occurred due to structural damage of the lining of the oviduct.

When considering the routine use of a procedure such as the dye test in cattle, it is of importance to know its effect on the subsequent fertility and carcase value if slaughter is decided of the animals used.

Of 5 cows inseminated 13 to 60 days after repeated dye tests 4 conceived to the first insemination and are now pregnant. This demonstrates the P.S.P. dye test does not appear to have any detrimental effect on future fertility. On the other hand, 7 animals sent for slaughter within periods ranging from 3 to 45 days after the carrying out of the dye test did not reveal any apparent genital tract infection, peritonitis, adhesions or cystitis and all were passed fit for human consumption.

However, it is obvious that there are still areas of unclear diagnosis between the clear-cut situation of patent oviducts and that of bilateral permanent occlusion. If as in the human the passage of dye through the oviduct could be visualised then a much more meaningful diagnosis would be achieved. Endoscopy was obviously an approach to be considered.

SECTION 3

E N D O S C O P Y

SECTION 3

ENDOSCOPY

Introduction

In the previous section results indicated a wide variation in the time taken for the dye to pass through the oviduct to escape at the ovarian open end. The reasons for these variations were not understood. Furthermore, none of the many reports on this test in cattle has demonstrated that the dye actually passes through the oviducts escaping into the peritoneal cavity.

Endoscopy if carried out successfully would afford direct visualisation of the genital tract as well as demonstrate whether it might elucidate the delay in the passage of dye along the fallopian tube which apparently occurs in some cows, as well as demonstrating whether the dye actually passes out through the oviduct into the peritoneal cavity. It was therefore decided to undertake this procedure in some of the experimental cattle.

Direct visualisation of the genital tract and andexa by endoscopy is now routinely used in human medicine as a diagnostic tool and as an aid to tubal surgery. Golditch (1971) reported that endoscopic visualisation when screening for oviduct patency using hysterosalpingography eliminates the sizeable incidence of false results.

One of the earliest reports of its use in veterinary medicine was that of Kelling (1902) who viewed the abdominal organs of a dog with a human cytoscope. The major setbacks of the earlier instruments was the excessive heat production at the source of the light on the endoscope, thereby limiting its use. However, with

the advent of modern high intensity fibre light optics utilising an external light source which overcame this problem it has been increasingly used.

Megale, Fincher and McEntee (1956), Dzuik, Dönker, Nicols and Peterson (1958), Lamond and Holmes (1965), Baker (1966) and Gangwar (1968) used indwelling plastic cannulas inserted in the flank for repeated endoscopic observations of the genital tract in the cow but found the formation of obstruction in the cannula with plugs of peritoneum, clots and fibrin to be a major hindrance.

Megale et al. (1956) also attempted endoscopic visualisation of the genital tract in cows through a puncture of the vaginal fornix and reported that this method was not totally satisfactory for cattle as it did not allow for a satisfactory examination of the anterior portions of the tubular genital tract. They also investigated endoscopic visualisation of the internal genital organs of female cattle via a puncture through the flank and reported that in most cases both ovaries, oviducts and uterine horns could be examined from a single puncture site.

Baker (1968) further modified the intravaginal technique for ovarian examination in the cow by making a 4-5 inch incision in the dorsal vaginal wall and bringing the ovary via the incision into the vagina which was distended with a speculum and illuminated with the light from a headlamp. This technique allowed up to 4 successful visualisations per cycle in an individual cow replacing the ovary in the normal position after each examination, with no apparent deleterious effects on ovarian function and subsequently found a 50% conception rate to first service.

Mariana (1969) described the paralumbar approach for

endoscopic examination of the ovary in cattle to be a "quick, simply, precise and undamaging technique" for following the ovarian activity of the same animal.

Wishart and Snowball (1973) further modified the flank approach for ovarian examination. Through one flank incision they introduced the endoscope and through an incision in the other paralumbar foss, a Jacobs-Palmer forceps via a cannula to grasp the mesovarium ligament to rotate the ovary allowing its entire surface to be studied in detail. They reported that insufflation of the abdomen is necessary only in the very fat subjects and with this there were no cardiac complications as reported in women (Carmichael, 1971). They gave a detailed review of the surgical anatomy of the region and a brief discussion on the problems associated with photography.

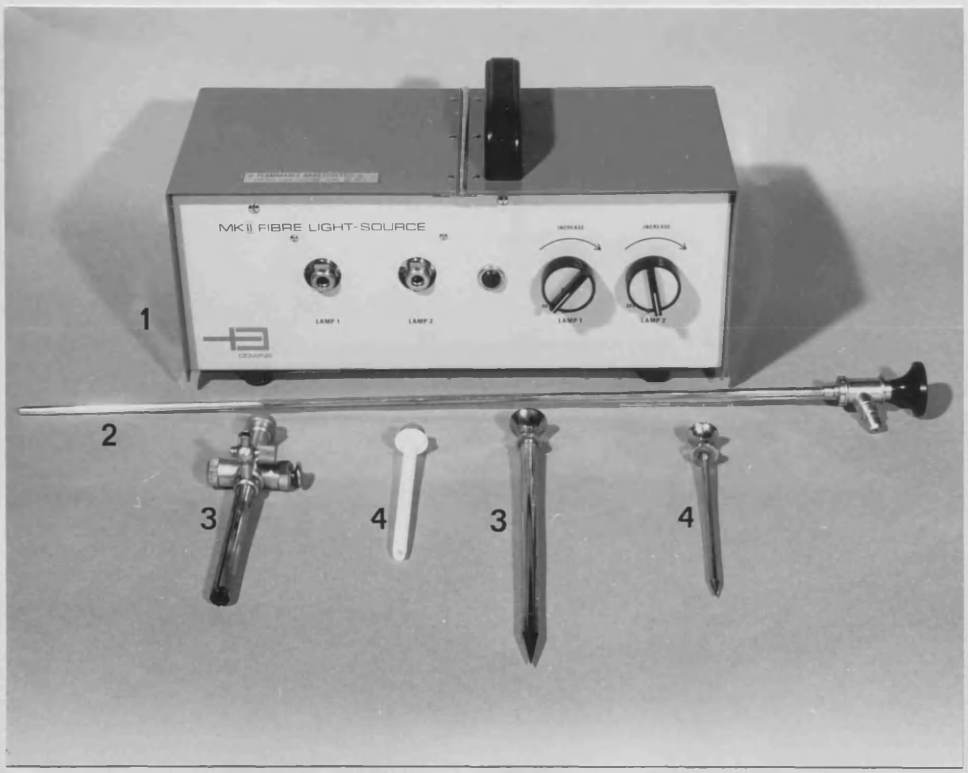
Schneider and Otta (1974), however, reported that insufflation of the peritoneal cavity was necessary for laparoscopic examination of genital organs in cattle and that 40-70 litres of laughing gas (N_2O) were suitable for this.

Wehrle (1974) also reported that insufflation of the abdominal cavity was required to provide the necessary space for laparoscopic examination and he used a mixture of 95% oxygen and 5% carbon-dioxide. He also reported success with the use of silicone rubber fistulas implanted in the flank and prevented occlusion of the lumen by the use of a watery suspension of dexamethasone. Only 1 out of the 15 fistulas implanted slipped out of the wound due to a local infection 15 days later, and the longest time they were in place was 55 days.

Other methods employed for direct visualisation of the

genital tract include laparotomy as was used by Umbaugh (1949) to observe the events occurring at ovulation in the cow and to collect ova for ovum transfer studies in cattle. Schultz, Fahning and Graham (1966) devised a new piece of equipment, the "Minnesota Laparotomy Apparatus" which allowed a much better exposure and much more room to work on the genital tract.

FIGURE 10



Equipment used in endoscopy

Materials

Cows and heifers of various ages, breeds and sizes. In each case the stage of the oestrous cycle was known.

Equipment

Some of the endoscopy equipment is shown in Fig. 10.

1. Downs Mark II fibre light source projector with a range of 200-250 volts.
2. Limina telescope with forward oblique vision of 180°.
3. Metal trocar with cannula.
4. Metal trocar with plastic cannula for second puncture.

The remainder of the equipment used for endoscopy is as follows:

1. Fibre light cable conducting light from the projector to the telescope.
2. Scalpel blade and handle.
3. Cotton wool and surgical swabs.
4. Mayo needle holder.
5. ½ circle cutting needle.
6. Monofilament nylon suture number 2.
7. 1½" 18 gauge hypodermic needles.
8. 2" 14 gauge hypodermic needles with short bevelled point.
9. 4" 16 gauge paravertebral nerve block needles with short bevelled point.
10. 2% Xylocaine.
11. Tilted cattle crush.

FIGURE 11

Equipment used for photography

Equipment for photography is shown in Fig. 11.

1. Phototelescope with distal electronic flash tube.
2. Cannula for (1).
3. Flash generator 200-250 volts range.
4. 35 mm Miranda sensomat RS camera fitted with an Exacta lens for endoscopic photography.

In addition the following equipment was used:

1. Synchronising cable.
2. Flash cable.
3. Kodak high speed Ektrachrome colour film EH 135-20.

Methods

Food was withheld from all animals for 24 hours prior to operation and water for 12 hours. A cattle crush was used to restrain the animal and the crush was tilted so that the animal's hind legs were elevated 8 inches above the level of the forelegs. The animal's tail was tied to the halter to prevent it from getting in the way during the entire procedure. An area approximately 3 inches cranial to the tuber coxae, and approximately 3 inches below the transverse processes of the lumbar vertebrae on either flank was clipped and shaved. The area was cleaned with cetavlon and warm water, dried and povidone iodine applied.

Anaesthesia

Two methods for anaesthesia were used.

1. Paravertebral nerve block

The animal's back was clipped 1½ inches away from the midline from the last thoracic vertebra to the 5th lumbar vertebra on either side. The area was cleaned and disinfected as described for the paralumbar fossa.

The spinal nerves T13, L1, L2 and L3 were blocked. Small areas of desensitised skin are produced at all sites of insertion of the paravertebral nerve block needle by injecting about 2 cc of Xylocaine subcutaneously with an 18 gauge 1½" hypodermic needle.

Nerve block for the thirteenth thoracic spinal nerve

The paravertebral nerve block needle is inserted between the 13th thoracic rib and the transverse process of the 1st lumbar vertebra approximately 1½-2" away from the midline. The needle was directed caudally to contact the anterior edge of the transverse process. It was then withdrawn slightly and redirected cranially

and slightly outwards until it penetrates the transverse ligament between the transverse processus. About 7 cc of Xylocaine is injected at this site to block the ventral root of the 13th thoracic spinal nerve. The needle was withdrawn and 7 cc of Xylocaine deposited above the transverse ligament. A further 4 cc of Xylocaine was deposited as the needle was withdrawn, and to prevent air pockets forming subcutaneously the skin was pressed down. At any time during the insertion or the withdrawal of the needle 1-2 cc of Xylocaine were deposited if the animal flinched.

Block for 1st lumbar nerve

The paravertebral nerve block needle was inserted between the transverse process of the 1st and 2nd lumbar vertebrae approximately 2 inches away from the midline and directed cranially to hit the caudal edge of the transverse process of the 1st lumbar vertebra. The needle was withdrawn slightly and redirected slightly caudally and downwards and 7 cc of Xylocaine deposited on either side of the transverse ligament. Again on withdrawing the needle about 4 cc of Xylocaine was deposited and 1-2 cc deposited at any time if the animal flinched.

Block for the 2nd and 3rd lumbar spinal nerves

The procedure was the same as for the 1st lumbar spinal nerve.

At any nerve block not more than 20-25 cc of Xylocaine was used.

Method 2

An 18 gauge 1½" hypodermic needle was inserted at the site of incision and Xylocaine was infiltrated in the area layer by layer when withdrawing the needle. The needle was directed dorsally,

centrally, caudally and cranially and anaesthetic deposited at all the tissue layers. About 5 cc of Xylocaine was deposited subcutaneously. Not more than 20 cc of Xylocaine was used.

At this point the rectum was emptied of faeces mechanically.

With either of the methods used for anaesthesia the effectiveness was checked by pricking the site of incision with a hypodermic needle.

A 14 gauge 2" needle was inserted at the site of incision on the right flank until it just penetrated the peritoneum when air was heard rushing in the peritoneal cavity. The needle was kept in placefor about 5 minutes to create a pneumonoperitoneum. This created a space between the superficial layer of greater omentum and the peritoneum, making it easier to introduce the trocar and cannula into the peritoneal cavity without getting into the omentum and the associated viscera. The thickness of the body wall was defined by the length of the needle inserted to create the pneumonoperitoneum. A small skin incision approximately 2 cm long was made in the middle of the prepared site on the right paralumbar fossa in a dorsoventral direction. The trocar and cannula are inserted inwards until the peritoneum was punctured, judged by the length of the trocar inserted. The trocar and cannula were then directed caudally and slightly downwards.

The trocar was removed keeping the cannula in place by holding it against the body wall and more air rushed in. The endoscope was inserted through the cannula directed caudally and slightly ventrally close to the peritoneum on the right body wall, towards the pelvic inlet. Caudal to the caudal duodenal flexure

and the caudal margin of the greater omentum the endoscope was directed towards the left body wall.

At this level the uterine horns and parts of the small intestines namely part of the ileum and jejunum were seen lying more ventrally. It was possible to see both the uterine horns, the body of the uterus, both ovaries and oviducts and their attachment to the broad ligament in most of the cows.

Approach through the left flank was carried out in animals where it was not possible to view all parts of the genital system through the right flank. Here the trocar and cannula were inserted in a slightly different way. As a pneumonoperitoneum was already present from the right flank approach, there is no need to repeat inserting the needle to let in air. The trocar and cannula were inserted inwards and after penetrating the peritoneum redirected caudally and slightly dorsally. The already present pneumonoperitoneum, the 24 hour starvation and the elevating on the hind quarters by the positioning of the crush reduced the possibility of puncturing the rumen accidentally. The trocar was removed and the endoscope was directed caudoventrally between the body wall laterally and rumen and the superficial layer of the greater omentum medially, until the caudal margin of the greater omentum was passed. The endoscope was then redirected towards the right, ventral and caudal to the right tuber coxae and on withdrawing it the right ovary and the uterine horn were the first part of the genital tract to be seen. Again the whole of the internal genital tract could be seen with careful manipulation of the endoscope.

Photography

Photographs were taken of the internal genital organs in situ

with the phototelescope and the camera connected to the various equipment necessary for generating the flash and synchronising it with the camera.

Suturing technique

After completion of the examination of the tract the endoscope was removed and the flanks of the animal were pressed to remove some of the air from the peritoneal cavity and then the cannula was removed. Only the skin incision was sutured with a single interrupted horizontal mattress suture using a number 2 monofilament nylon.

Post-operative treatment

The animal was given intravenous injection of Terramycin Q 50 mg/ml for 3 days, the amount depending upon the size of the animal. The sutures were removed after 6 days.

Results

The results of the endoscopic examination carried out are shown in Figs. 12, 13, 14 and 15.

FIGURE 12

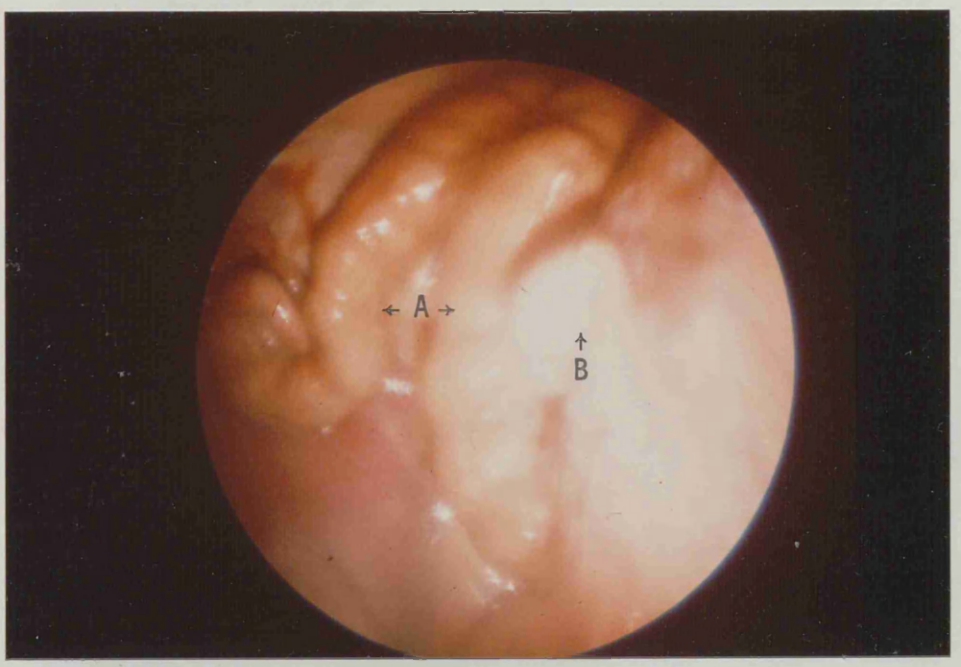


Figure 12 shows the whole genital tract as seen via the endoscope.

Note the uterine horns (A) hanging down at the pelvic brim and the left ovary (B) just at the pelvic brim.

FIGURE 13

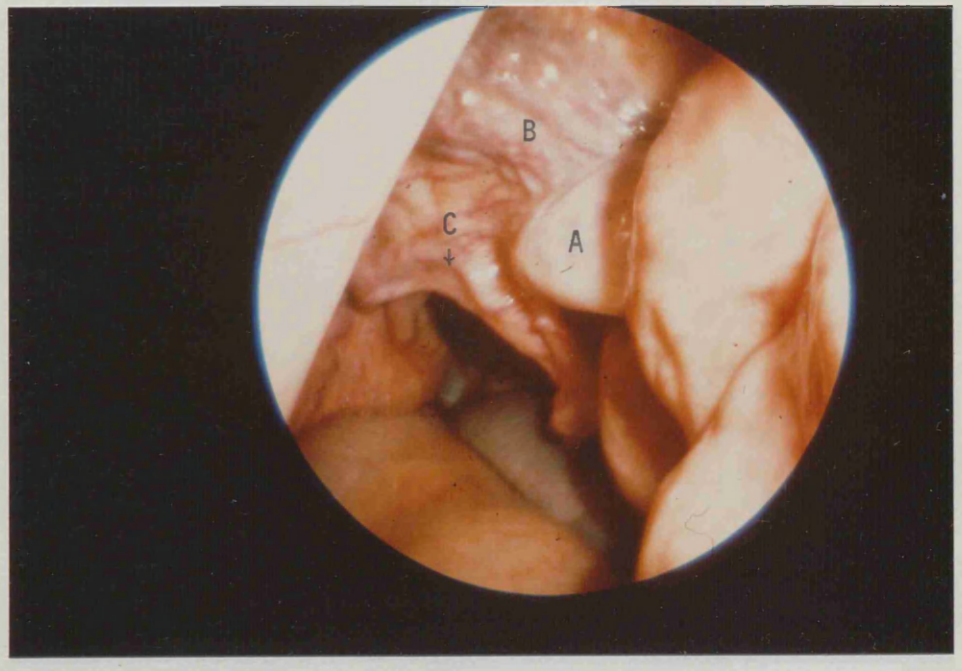


Figure 13 shows a right ovary (A) partly hidden.

Note the association with the broad ligament (B) and the oviduct (C) in the ovarian bursa lying distant from the ovary.

FIGURE 14

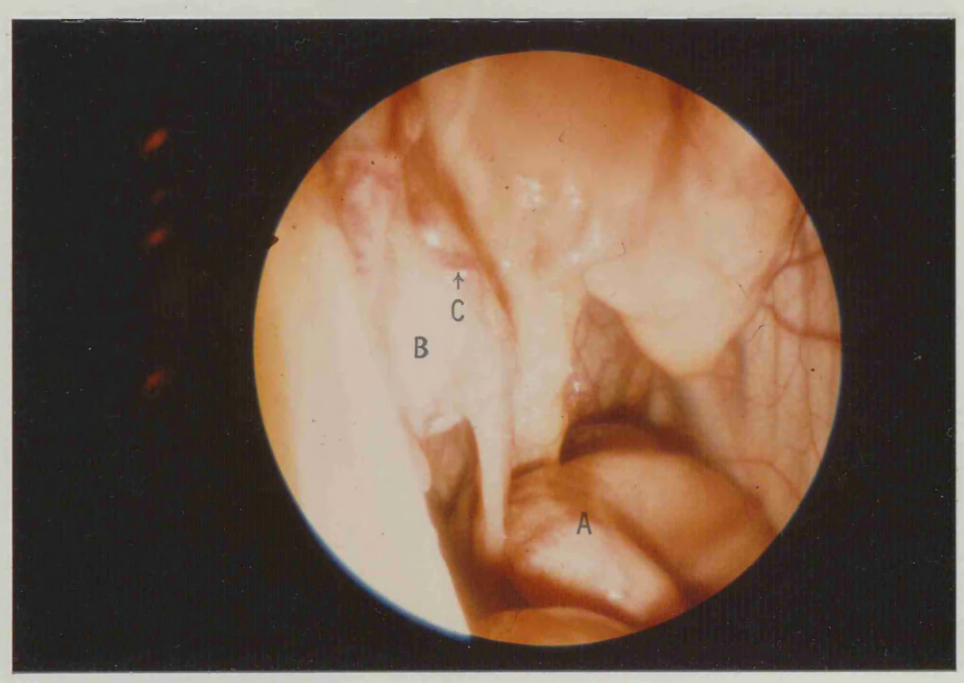


Figure 14 shows a coiled uterine horn (A) distended with P.S.P. dye.

The ovary is hidden from view by the ovarian bursa (B). Note the distended oviduct (C).

FIGURE 15

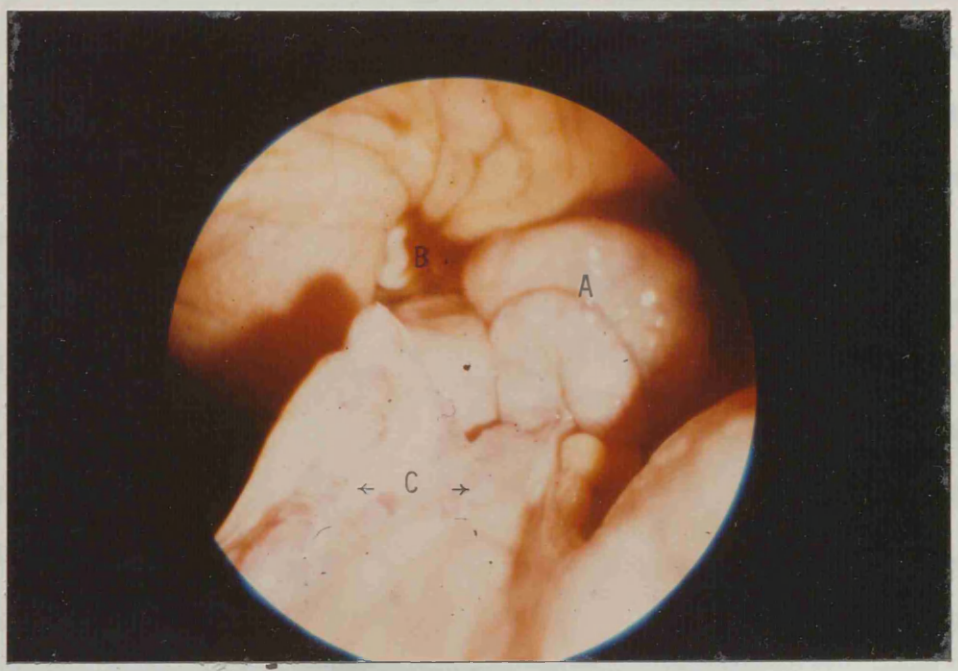


Figure 15 shows a coiled uterine horn distended with the P.S.P. dye (A).

Note the pooling of the dye near the uterine horn (B) and mottling of the adnexa with the dye (C).

Discussion

There were several initial technical problems that had to be overcome before successful visualisation of the genital tract in the cows could be carried out using an endoscope. In human patients endoscopy is carried out under general anaesthesia with the patient on her back, tilted at the required angle to allow easy access to the reproductive tract. However, when an attempt at endoscopic examination of the female genital organs was made in cattle under general anaesthesia it was found that the weight and size of the abdominal contents made it impossible to view the pelvic region at all. Therefore endoscopy was carried out with the cow restrained in a tilted cattle crush under local anaesthesia. This approach proved reasonably successful. By trial and error it was found that tilting the cattle crush at an angle of approximately 30^{o} allowed access to the reproductive tract and this did not result in any respiratory embarrassment to the animal. Either paravertebral anaesthesia or local infiltration were carried out initially and it soon became apparent that of thw two methods, local infiltration was much the better. Local infiltration was found to be a simple rapid procedure requiring little prior preparation (minimum amounts of anaesthetic and little delay between time of application and commencement of the operation). In a few cases where the animal was hyperexcitable epidural anaesthesia was sufficient to control any violent movements.

If the abdomen was entered without prior introduction of air, it was found that the bulk of the abdominal viscera was such as not to allow the required manouverability of the endoscope. This situation was improved slightly by withholding food from the

cows for 24 hours prior to operating. In addition immediately prior to carrying out endoscopy, mechanical removal of the faeces from the rectum also helped to increase the space in both the abdominal and pelvic cavities. When examination was attempted by approaching from a left flank incision a major setback was the frequency with which accidental perforation of the rumen occurred. If, however, a preliminary check was made as to the exact position of the caudal border of the rumen, hence making sure that the trocar and cannula was inserted well behind this border, then perforation was avoided. However, a right flank approach was found to be easier. In many of the cases examined the rumen occupied the greater part of the paralumbar fossa and in these cases such prior precautions were of no avail. Another difficulty was experienced in those cases where accidental perforation of the greater omentum occurred. This resulted in air becoming trapped within the omentum forming a balloon-like obstacle to the entry of the endoscope.

However, a clear unveiled view of the uterus and ovaries was obtained in those cases where initially air was allowed to enter the peritoneal cavity via a large hypodermic needle, the endoscope was then introduced via the cannula and worked downwards in the direction of the pelvic inlet lifting aside the greater omentum as it passed. With experience both horns and both ovaries could be exposed and viewed relatively easily in the majority of cases. In most cases the whole tract could be viewed from a right flank incision. In a few cases, however, e.g. in very fat cattle, both right and left flank approaches had to be carried out in order to see both oviducts.

Initially some difficulty was experienced in recognising

the various parts of the reproductive tract. This applied mainly in recognition of the ovaries which were enveloped by the ovarian bursa and recognition was found to be especially difficult in those cases where there were no obvious recognisable features present on the ovaries, such as corpora lutea and/or large follicles. However, if the ovarian ligaments were identified initially and their associations with the broad ligament recognised this resulted in pin-pointing the position of the ovaries. The ovarian bursa could then be pushed out of place to reveal the ovary for inspection.

On introduction of dye into the uterus the distention which occurred was easily recognised. The presence of the dye caused coiling of the uterus which was especially marked around the tips of the uterine horns. The uterus tended to remain coiled throughout the endoscopy procedure lasting for at least 15 minutes. In all the cases examined by endoscopy in the work described in this thesis there was a delay in dye in the uterus gaining entry to the patent oviducts. This period varied from 4 to 7 minutes. Uterine contractions were not obvious during this time lapse but this may well have been due to the fact that all abdominal contents were in constant motion. The first sign of things happening within the oviducts was the escape of air from the ovarian open end or ends. This was accompanied by straw coloured fluid, 4 to 5 minutes later the dye was seen to flow from the fimbriated end of the oviduct as a fine jet of fluid and indeed in most cases was first noticed as dye staining the adnexa and the ovaries and collecting in the folds of the broad ligament. These findings tend to suggest that there is an initial build-up of pressure within the uterus most probably brought about by uterine contractions followed by the forcing out of the

material occupying the lumen of the oviducts and then the dye appears. This also suggests that the passage of the dye along the oviducts is not a simple easy flowing motion but requires a certain amount of pressure to force its way upwards. Ballooning of the ovarian end was easily recognised and the red coloured dye was easily seen through the relatively thin walled infundibulum. However, the dye was never positively identified either by colour or by the stretching of the relatively thick muscular walls of the isthmus. Indeed even when blue coloured dye was used this was not able to be identified within the lower part of the oviduct. In the few cases examined in the study described here with total occlusion of the oviducts it was found that the dye did not enter the oviduct at all,at least there was no ballooning of the infundibulum. This, however, for the reasons just described would not necessarily mean that the oviduct was blocked for its whole length. Therefore from this point of view endoscopy under the circumstances in which it was carried out in this thesis would not positively identify the actual area of occlusion, unless it occurred in the upper thin walled infundibulum. In one of the animals studied it was known from a previously carried out dye test that she most probably had occlusion of one of her oviducts. When endoscopy and another dye test were carried out at the same time it was seen that though air and some straw coloured bubbles were seen to be escaping from the ovarian end of the oviduct in question there was no evidence of the dye escaping from the ovarian end.

At the conclusion of this procedure the cow was slaughtered and the dye was seen to be present in the whole length

of the tube to within a few cms of the end of the tube. Additional pressure applied to the tube did not express the dye. This would seem to suggest that in this particular case the blockage at the end of the tube was partial and although allowing a fine stream of air bubbles to pass through, no identifiable amounts of dye were able to be passed. Added to this was the fact that in this animal uterine rupture had not occurred even with the additional amount of pressure required to even force through the few air bubbles seen.

Of the animals in which endoscopy was carried out, 3 were subsequently served and all are now known to be pregnant. Two others were killed 7 to 14 days afterwards and post-mortem examination did not reveal any signs of obvious peritonitis or adhesions either at the site of incision or in any area of the genital tract. This would suggest that as an aid to diagnosis along with the P.S.P. dye test endoscopy can prove a very useful method, especially in the unilateral obstruction case in which the dye test on its own can lead to confusing results.

Finally, under certain circumstances, where only partial blockage is present endoscopic examination along with some technique for removal of such blockage could be envisaged as a possible means of treatment in certain infertile cows where future production was of great importance.

GENERAL DISCUSSION

There are many cases of infertility in cattle where the diagnosis is not reached in the living animals. Many of these are kept for some time with the hope of establishing pregnancy. During this time too much expense is incurred, costs of veterinary services, feed maintenance, routine management procedures, e.g. vaccinations and repeated inseminations. Although the incidence of permanent bilateral occlusion of oviducts in cattle appears to be low, if these cases are diagnosed early enough, they can be slaughtered, thereby reducing at least some of the expenses incurred where no economic returns are forthcoming.

The phenosulphonphthalein (P.S.P.) test appears to be a suitable method for diagnosis of permanent bilateral occlusion of oviducts in cattle for various reasons. The foremost advantage is that the diagnosis is accurate and can be reached by, at the most, three tests. The fact that the P.S.P. dye test does not affect the value of the carcase makes it suitable for both dairy and beef cattle. The application of the test does not require expensive equipment. The equipment is usually available in most large animal practices and the dye is easily obtainable and the preparation of the solution simple. Furthermore, with proper planning the test can be carried out simultaneously on a group of cows in approximately the same time as would be necessary for the test in one cow. Thus when examining the infertility cases in a herd, those with no obvious reasons for infertility can be examined for oviduct patency with the P.S.P. dye test on the same visit.

The pregnancy rate found in the studies described here, subsequent to the dye test in the small number of animals inseminated seem to indicate that this test does not reduce the future fertility of the cattle. However, before any definite conclusions can be drawn on this aspect, the test would have to be applied to larger numbers of cattle and the subsequent fertility evaluated.

An interesting observation with the P.S.P. dye test was that in some of the cattle in which the initial appearance of the dye in urine was delayed on the first test, on repeating the test on two or three occasions the dye appeared in the urine well within the time recorded in cows with bilateral oviduct patency. This effect of repeated dye tests could possibly result in re-establishment of normal fertility and this is an area that deserves investigation.

There could be cases where oviduct patency is demonstrated but pregnancy does not follow. In these cases oviduct pathology could be suspected, e.g. salpingitis, adhesions in the lumen for which there are no known methods of diagnosis and treatment. Consideration should be given to the investigation of the possible therepeutic value of transuterine infusion of oviducts with antibiotics.

Endoscopy, though for a long time to come would basically be a diagnostic tool in research and teaching organisations, could be helpful in explaining some cases of infertility where the oviducts are patent and rectal examination fails to reveal mild paratubal pathology, e.g. adhesions, which could cause infertility.

From this study an important area for further research which could be beneficial in cattle reproduction would appear to be the investigation of the cause of endometrial rupture that occurred

during the *in vitro* transuterine insufflation of oviducts. Successful endoscopic examination of the endometrium in living animals has been possible recently (Leidl and Schallenberger-Pottiez, 1976) and such an approach could be used to examine the site of rupture after the insufflation test in living cattle. Virology and bacteriology of endometrial biopsy material could reveal pathogens in these cases and perhaps electron microscopy could possibly reveal as to how these organisms predispose to endometrial rupture.

It is obvious that at present the real value of the P.S.P. test in cattle lies in the diagnosis of blocked oviducts but further investigations may yield interesting results. There is a very urgent need for economic reasons to investigate the cause of sterility in these unexplained cases, and then to devise ways and means to diagnose such conditions early in the living animal. It is only when these conditions are understood and diagnosed that one can investigate possible ways of prevention and treatment, and achieve a more efficient cattle production.

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