SOME ASPECTS OF INFERTILITY IN THE BRITISH HIGHLAND CATTLE

BY NG IN HOOI DVM

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LIST OF ABBREVIATIONS

BHC	BRITISH HIGHLAND CATTLE
GnRH	GONADOTROPHIN RELEASING HORMONE
ACTH	ADRENOCORTICOTROPHIN HORMONE
ET	EMBRYO TRANSFER
AI	ARTIFICIAL INSEMINATION
ANOVA	ANALYSIS OF VARIANCE
N	NITROGEN
P	PHOSPHORUS
K	POSTASSIUM
AP	ALKALINE PHOSPHATASE
mHz	MEGA HERTZ
FIG	FIGURE
nm/l	NANAMOLE /LITRE
%	PERCENTAGE
SD	STANDARD DEVIATION
AV	AVERAGE
kg	KILOGRAM
d	DAY
cm	CENTIMETER
CL	CORPUS LUTEUM
BVD	BOVINE VIRUS DIARRHOEA
IBR	INFECTIOUS BOVINE RHINOTRACHEITIS

DECLARATION

I, Ng In Hooi, do hereby declare that the work presented in this dissertation is original ,was carried out by me and has not been presented for an award of a degree in any other university.

Signature : .....
NG IN HOOI

Date: 30/11/92

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SUMMARY

This study investigated some aspects of infertility in British Highland Cattle on two farms in Scotland. In farm 1, the study involved 48 head of post-partum cows which were examined, monitored for fertility and recorded in a computer spread sheet using a commercial spreadsheet "Microsoft Excel". This investigation also involved the study of chromosomal defects and examination of reproductive tracts using rectal palpation, plasma progesterone concentrations and ultrasonography.

A second herd of animals, on farm number 2 near Oban, Argyll, which had reproductive problems in growing heifers was also examined for other aspects of fertility. Examination of abattoir specimens of the reproductive tracts from culled unproductive animals was carried out. Chromosomal analysis was undertaken on both of these farms when the initial blood culture revealed the existence of Robertsonian translocation in farm 1.

On the whole, the reproductive parameters in farm 1 showed a slight decline in fertility compared with accepted standards. The results implicated certain individual cows which had contributed to this reduction in fertility. This could be seen in the wide variations of the results obtained. Thus, in order to improve the fertility of the herd, it was necessary to identify these problem cows and consider culling them as culling due to infertility was almost negligible on the farm.

The causes of infertility were complex as no simple cause was identified. This study could only rule out likely causes through the process of elimination and to concentrate on the potential areas that could be the

contributing source of this infertility problem. Specific disease screening of the bulls was recommended, especially when natural mating was practiced. Further studies in this area could elucidate, and may help to identify, the cause of infertility. As there was no simple cause of the infertility in herd 1, various ways of improving the fertility through good management, nutrition and introduction of superior genetic materials by the use of AI or even ET is recommended. Thus, computerisation of the breeding records would enable fertility analysis to be carried out promptly and action lists produced to assist in the management decision.

On farm 2, the majority (92%) of the uterine cultures of the reproductive tract from the abattoir specimens harboured no bacteria. About 67% of these tracts showed some form of gross morphological abnormality involving the tubular genitalia. However, histological sections of the endometrium revealed no obvious abnormalities.

Detailed chromosomal investigation was carried out on 69 animals. This showed that 11.36% and 12% of cattle on farms 1 and 2 respectively were found to be heterozygous for a Robertsonian translocation, identified as involving numbers 1 and 29. Studies of the pedigrees of the last 5 generations of the positive cases were carried out to identify the source of inheritance. It is suggested that <u>de novo</u> production of the genetical aberration could have occurred some generations ago in the breed. It was found that the defect did not have any apparent affect on fertility in the present study. This was the first time that the 1/29 Robertsonian translocation has been found in this breed.

SOME ASPECTS OF INFERTILITY IN THE BRITISH HIGHLAND CATTLE

CHAPTER ONE

1.0 INTRODUCTION

The British Highland breed (Plate 1,2) is one of the oldest purebred beef cattle, which has been in existence for many centuries (Rouse, 1970). It is not derived from any other breeds and is one of the hardiest and thriftiest beef cattle in Scotland, surviving fairly adverse conditions. They are not housed and do not require any expensive buildings, feeding or labour. The cattle are also known for their attractiveness, longevity and quality of beef. There has been a renewed interest in recent years in the reproductive performance of this breed due both to the increased demand for the breed and in its purity as a rare breed.

Attention has been focussed on conception rate, calving intervals, abortion, mortality and culling rates. The ideal reproductive index is a maximum of 85 days from calving to conception in order to maintain a 12 month calving interval (Manns and Richardson, 1976) so as to achieve not more than 1.8 services per conception (Morrow et al, 1966a). Prolonged calving interval decreases the production per cow through fewer calves born per cow per year as well as increased culling rate. In addition, economic losses are also due to additional breeding costs especially when AI is used, as well as increased veterinary services, medication and replacement costs. It has been estimated to cost about £0.50 per cow per day to feed on farm 1. Thus, with a calving interval of 386 days, there would be a loss of about £10.50 per cow. In a herd of 50 breeding cows, there would be accumulated loss of about £525 per year on feeding costs alone. In the United Kingdom, in recent years been reported that losses due to infertility was £290 million (Peters and Ball, 1987).

PLATE 1 BRITISH HIGHLAND COW

PLATE 2 BRITISH HIGHLAND BULL

The term infertility covers a wide variety of meanings. It may denote a slight depression of breeding efficiency or sub-fertility, a prolonged inability to breed or even sterility. Infertility in cows presents one of the most difficult investigative problems because it is rarely due to only one factor. The complex and multiple factors responsible for the infertility contribute largely to the difficulties that beset the whole field of investigations. The basic causes of reproductive problems in a herd are not always apparent and genetic, management, nutritional, diseases, and pathological factors might be involved. Therefore, a practical investigation as to the causes of infertility in a herd is difficult. This is because one has to work within the constraints of the farm policy, time, costs as well as obtaining good accurate data. In developing the study of infertility among the British Highland Cattle (BHC), it was necessary to draw inferences from the breeding records accumulated over the last six years in order to have a clear picture of the extent of the manifestations of the infertility problem. Clinical tools were used to help in the investigation and to monitor the clinical performance of the herd. A study of such a nature did not lend itself to controlled experimental procedures by virture of the need for economic reason. Finally, the problem of infertility might likely come to an end even before the result of the investigation was finalised because pragmatic steps would be taken to remedy the situation to prevent further loss. There have been few studies on beef herd fertility and no detailed comprehensive studies are yet published on BHC fertility. It would be reasonable to compare the results with dairy cattle in which extensive work has been carried out.

The study on the chromosomal aberrations was carried out in the light of various reproductive problems on

farm 1, in order to determine its effect on fertility. Chromosomal aberrations have been associated with infertility of various types in cattle. Apart from major anomalies such as XXY in infertile males of various species (Logue et al, 1979) the most frequently reported structural abnormalities of chromosome in cattle were chromosome translocations of the Robertsonian type. Robertsonian translocations or centric fusions are the fusion of two telocentric chromosomes at the centromeres to form one metacentric chromosome (Robertson, 1916). This has also been used to describe the centromeric fusion of two acrocentric chromosomes which was produced when a break occurred in the centromere of both chromosomes where the long arms fused together to form a metacentric chromosome containing virtually all the genetic material. The short arm fragments were then fused to form a minute acentric chromosome which was lost during subsequent cell divison (White, 1957).

The 1/29 translocation had been detected in almost 50 breeds of cattle worldwide (Kovacs, 1989). It was initially reported by Gustavsson and Rockborn (1964) in the Swedish Red and White breed. Subsequently it was reported in Europe (Amrud, 1969) and the United States of America (Herschler and Fechheimer, 1966). Since then the translocation had been identified in South America (Pinheiro et al, 1979; Moraes et al, 1980), Japan (Masuda et al, 1975), and Asia (Fischer, 1979). The frequency of this abnormality varied with the breeds. It was found to have a particularly high incidence in several breeds of beef cattle (Harvey, 1972; Harvey, 1976; Gustavsson, 1979). Robertsonian translocation of the 1/29 type was found in pure-bred British Friesians only recently and was presumably to have formed de novo (Wilson, 1990) a relatively short time previously.

However this translocation has not been identified in the British Highland breed.

The objective of this study was to investigate some aspects of infertility in British Highland Cattle utilising the post-mortem examination of reproductive tracts using various clinical tools such as chromosomal analysis, rectal palplation, plasma progesterone assays and ultrasonography.

CHAPTER TWO

2.0 REVIEW OF THE LITERATURE ON THE CAUSES OF INFERTILITY

Infertility is the inability to produce viable young within a stipulated time, whereas sterility is a permanent condition preventing reproduction. The factors that exert their influence on fertility are environment, management, nutrition, hormones, genotype and disease (Jainudeen and Hafez, 1990). However, there has been a noticeable change in the causes of infertility in cattle over the last two or three decades. This is because some diseases have largely been eliminated with the introduction of artificial insemination and the use of new diagnostic tools. Examples are brucellosis and tuberculosis. Non-specific infections due to opportunist pathogens derived from poor management of a herd are still by far the greatest cause of infertility.

It is proposed to examine the various causes of cattle infertility under four main headings: anatomical factors, functional abnormalites, management and disease.

2.1 ANATOMICAL FACTORS AFFECTING FERTILITY

Acquired abnormalities have been more frequently encountered than congenital ones, the frequencies in one study being 9.65% and 0.3% respectively of 2000 genital tracts which were examined from abattoirs (Kessy, 1978). These latter abnormalities affect individual cows or heifers and do not have a major influence on herd fertility.

2.1.1 CONGENITAL ANOMALIES

There are various developmental defects of the Mullerian ducts which lead to anomalies of the vagina, cervix and uterus. The affected animals show normal cyclic behaviour because the ovaries develop normally. One of the most common defect of the female tubular organs is the persistence of the hymen. In this condition there is an accumulation of cyclic secretions in front of the obstruction and the animal cannot be inseminated artificially. This is associated particularly with white Shorthorn heifers and has thus become known as 'white heifer disease'. The condition is due to a sex-linked recessive gene with linkage to the gene for white coat colour. (Arthur et al, 1989) Partial or segmental aplasia of the Mullerian ducts as in **uterus unicornis** where only one horn has a lumen are also found. In **uterus didelphys** each uterine horn connects with the vagina by a separate cervical canal. The animal conceives normally, but may show dystocia due to a fetal limb entering each cervical canal. Similarly, in animals with **double os uteri externum** or **dorsoventral postcervical band**, there may be difficult births or expulsion of afterbirth.

2.1.2 EFFECTS OF 1/29 TRANSLOCATION AND EMBRYONIC DEATH

There are no visible effects on the phenotype in either heterozygous or homozygous carriers of the 1/29 translocation in cattle (Gustavsson, 1969). Genetically balanced gametes occur during meiotic segregation when the homologous chromosomes are separated into one daughter cell and the translocation into the other. However, if one homologous chromosome segregates with the translocation, genetically

unbalanced gametes are produced, this mechanism being known as adjacent segregation. In other words, this type of segregation leads to formation of either disomic or nullisomic gametes. After fusion with normal gametes, monosomic or trisomic zygotes are created. These unbalanced embryos are not viable, thus resulting in embryonic mortality. This explains the drop in the fertility shown by an increased non-return rate (Gustavsson, 1969).

There is a correlation between chromosome constitution and infertility as reported by many researchers (Lyon and Meredith, 1966). It was believed that zygotic death was due to chromosome aneuploidy caused by malsegregation of the translocation products at meiosis. Trials have been undertaken to compare non-disjunction rates in heterozygous carriers with those of animals with normal chromosomal karyotype (Gustavsson, 1969; Pollock and Bowman, 1974). The level of non-disjunction is about 10% based on the criteria of doubling the incidence of secondary spermatocytes bearing one extra chromosome (Gustavsson, 1969; Logue and Harvey, 1978). Studies demonstrating the existence of unbalanced gametes in animals heterozygous for the 1/29 translocation have been carried out by a number of researchers (Gustavsson, 1969; Logue, 1977; Logue and Harvey, 1978; Popescu, 1978; Popescu, 1980; King et al, 1981).

The transmission of 1/29 translocation in a balanced state is according to the Mendelian ratio of 1:1 (Gustavsson, 1969). That is, the translocation is inherited in about half of the calves from a heterozygous parent (Gustavsson, 1969; Potter et al, 1979; Blazak and Eldridge, 1977; Kovacs and Csukly, 1980).

In heterozygous bulls, the libido and sperm characteristics were found to be normal with apparently normal fertility (Gustavsson, 1969; Moustafa et al, 1983). Only the spermatozoal concentration was slightly reduced. In another study with 12 heterozygous bulls unselected for fertility, there was a reduction in non-return rate at 28d and 56d of 4.85% and 7.02% respectively compared with normal bulls (Dyrendahl and Gustavsson, 1979). The sperm characteristics and libido were normal except for slight decrease in the concentration of spermatozoa. It has also been confirmed that a higher frequency of degenerated embryos or embryos in the process of degeneration was found in three heterozygous 1/29 carrier bulls bred to superovulated cows (Linares et al, 1980).

This decline in fertility from 30 to 60 days was confirmed in the Norweigan Red breed (Refsdal, 1976) and by several authors (Queinnec et al, 1974; Blazak and Eldridge, 1977; Kovacs and Csukly, 1980). This reduction in fertility in males and females appears to be because of an increase in early embryonic mortality (Gustavsson, 1969), due to the formation of trivalent structures during meiosis resulting in non-disjunction and unbalanced gametes produced which were involved in fertilisation but then subsequently died. His explanation has been confirmed by meiotic studies in animals heterozygous for the 1/29 translocation in which a certain percentage of unbalanced gametes existed (Gustavsson, 1969; Logue, 1977; Logue and Harvey, 1978; Popescu, 1978). It was shown by Logue and Harvey (1978) that aneuploidy existed in approximately 6% of secondary spermatocytes from bulls heterozygous for the 1/29 Robertsonian translocation. In embryonic studies in superovulated cows inseminated with semen from bull heterozygous for the 1/29 Robertsonian translocation, monosomy (Popescu, 1980) and trisomy

(King et al, 1981) embryos were recovered which would have been expected to cause a reduction of fertility in heterozygotes.

In cattle, it was found that the fertilisation rate was 85% and the embryonic survival rate between 9 and 26 days was 70% (Boyd et al, 1969; Wijeratne, 1973). Therefore, fertilisation failure occurred in 15% of cases, early embryonic death before day 26 was about 7.5% and loss of foetuses was about 8% (Boyd et al, 1969). Other workers have reported a much higher fertilisation rate (Bearden et al, 1956). Fertilisation rate was reported to be 90% and embryo survival rates up to days 8, 12, 16 and 42 were 93%, 56%, 66% and 58% (Diskin and Sreenan, 1980). This is in agreement with other findings that most embryonic death in cattle occurs after 8 days post-oestrus (Roche et al, 1981). Degenerative changes have also been observed to occur in some cattle embryos by 7 or 8 days post-oestrus (Ayalon, 1978; Linares, 1982; Maurer and Chenault, 1983).

2.1.3 ACQUIRED ABNORMALITIES

There is a high frequency of occurrence of lesions of the **uterine tube and adnexa** the frequency of which was reported to be 15.3% of the cows examined clinically (Carpenter et al, 1921). The most frequently observed lesions were between the ovary and the ovarian bursa. Web-like adhesions of the ovarian bursa were found in 62% of the slaughterhouse cattle (Edwards, 1961) although all these may not interfere with fertility. However, in bilateral cases, they may interfere with ovulation or impede sperm or egg transport through the oviduct. When the oviduct is occluded, there can be an

accumulation of secretions which causes hydrosalpinx. If these cysts become infected it produces pyosalpinx and intra-ovarian and periovarian abscesses may develop. Normally, the incidence of ovarobursal adhesions increases with age of the cow. It was reported to occur in cases of tuberculosis peritonitis and following enucleation of corpus luteum, rupture of ovarian cyst and mycoplasma infection (Hoare, 1969) and viral epididymovaginitis of cattle in East Africa. However, the most likely cause is puerperal infection due to ascending infection or perimetritis. It can also be induced by intrauterine infusion of Lugol's iodine in large volumes, particularly under pressure (Arthur et al, 1989). Diagnosis of ovarobursal adhesions using rectal palpation is done by holding the ovary between thumb and forefinger, and using the other three fingers to extend the ovarian bursa. Other examination of the ovaries are by laparotomy and the infusion of phenolsulphonphthalene (PSP) into the uterine lumen or horn (Kothari, 1977). The dye passes into the peritoneal cavity, is absorbed into the circulation and excreted by the kidneys into the urine where it produces a red or pink colour. There will be no discoloration of urine if the uterine tubes are occluded. The test is performed during the luteal phase of the cycle to avoid false negatives. However, a more accurate method of evaluating the uterine tube patency is by using gamma-irradiated starch particles in order to simulate the transport of the oocyte. The starch particles are similar in size to the ovum of the cow. There are instances where the oviducts which proved to be patent to dye were subsequently found to be abnormal as starch grains were unable to pass freely from one end of oviduct to the other.

2.2 FUNCTIONAL FORMS OF INFERTILITY

Functional forms constitute an important cause of infertility usually in individual animals. Most functional aberrations occur because of endocrinological abnormality which is associated with inherited factors, nutritional deficiencies or excesses, social influences and the stress of production.

2.2.1 NO OBSERVED OESTROUS

The ovaries of a cow in **true anoestrus** are quiescent. This may be due to insufficient release or production of gonadotrophins or the failure of the ovaries to respond which is unlikely. It is most frequently found in high-yielding dairy cows, first calvers, and beef suckler cows. There are a number of predisposing factors namely, season of calving, heredity, suckling effect and nutrition. Autumn calving herds tend to show true anoestrus because they are housed indoors and fed on preserved fodder (Oxenreider and Wagner, 1971). Beef breeds (36-70 days) generally take longer to resume post-partum cyclicity than dairy breeds (10-45 days). Non-suckling cows exhibit their first oestrus 10-33 days post-calving compared with suckled cows which did not return to oestrus until at least 98 days post-partum (Radford et al, 1978). This has be reported to be because suckling stimulates prolactin secretion (Karg and Schams,1974) which may reduce ovarian sensitivity to normal levels of plasma LH (Bartosik et al, 1967). This could also be due to suppression of GnRH secretion by low level of prolactin inhibitory factor (PIF) and hence decreased gonadotrophin production (Hafez, 1975). However, there is no direct correlation between high prolactin concentration and ovarian inactivity (Radford et al, 1978). High milk

yield has also an effect on postpartum ovarian activity (Oxenreider and Wagner, 1971) whilst others suggest that it is the result of body weight loss and nutritional deficiency.

Silent heat normally occurs during the first and second ovulations immediately post-partum (Morrow et al, 1966b). Behavioural oestrous activity is lowest at midday while the greatest activity occurs in the evening. Thus, it requires at least three periods of daily observations for optimum heat detection. The incidence of silent heats was 10.6% even when cows were examined four times a day (Hall et al, 1959).

2.2.2 OVULATORY DEFECTS

A number of **ovulatory defects** affect fertility in that either the oocyte is not liberated, liberated too late, or the oocyte has aged thus being incapable of normal development. These defects are due to endocrine deficiency or imbalance and mechanical factors. Ovulation is delayed or fails to occur if the quantity of pituitary hormone released is insufficient or its timing is incorrect. Ovulation may also be prevented if there is extensive adhesion of the ovarian bursa.

There is little information on the incidence of **delayed ovulation** associated with infertility. It is recommended that if ovulation has not occurred by 24 hours after service the cow should be reinseminated (Van Rensburg and de Vos, 1962). **Ovaries are defined as cystic** when a mature follicle is greater than 2.5cm in diameter. In 60% of cows, an ovarian cyst occurs before the first postpartum ovulation (Morrow et al, 1966b). However, they normally resolved spontaneously and do not cause aberrant reproductive function. At a later post-partum stage, ovarian cysts are an important

cause of infertility. They have been reported to vary from 5.6% to 47.4% in dairy cows (Kesler and Garverick, 1982). However, beef breeds are seldom affected. Endocrine abnormalities are considered to be the major cause. Any defect or asynchrony in the complex process of folliculogenesis and ovulation could lead to cyst formation. There are various suggestions that it could be due to deficient LH release (Nadaraja and Hansel, 1976), absence or reduced response to the preovulatory oestrogen peak, asynchrony of hormonal events (Zaied et al, 1981), defects within the ovary, elevated ACTH (Christian et al, 1965), high prolactin secretion (Hafez, 1975) or hypothyroidism (Eyestone and Ax, 1984). Ovarian cysts have been classified as either follicular cysts which are thin-walled or luteinized cysts which are thick-walled with luteal tissues. Cows with follicular cyst are usually nymphomaniacal but do not necessarily have elevated blood oestrogen concentrations (Dobson et al, 1977). Thus, it was concluded that the changes are likely to be due to an increased period of oestrogen domination (Dobson et al, 1977). The luteal or luteinized cyst results in a cessation of cyclicity activity. Diagnosis of cystic ovary is dependent upon the history, clinical signs, rectal findings, and progesterone concentration in the blood.

Spontaneous recovery can occur in 50% of the cows which develop cysts within 45 days of calving (Morrow et al, 1966a), whilst others have reported recovery only in 13-29% of cases (Bierschwal et al, 1975). The earliest method of treating cysts by manual rupture is no longer recommended because it might cause ovarobursal adhesions. Follicular cysts have been treated with GnRH or hCG with 90% and 76% response while 50% and 27% respectively conceived (Dobson et al, 1977). Over 80% of the cows treated with GnRH resumed normal cyclical

activity within 18-23 days after treatment (Kesler at al, 1978). Another most successful method of therapy is the PRID (progesterone releasing intravaginal device). This success is partly due to the fact that the signs of nymphomania abate within 24 hours as well as the fact that the cyst regresses. When the PRID is removed after 11 days, oestrus occurs followed by ovulation and CL formation. However, in cows with luteal cysts the treatment has been the use of prostaglandin F2 alpha. It was shown that 26 of 27 cysts had regressed after treatment with the majority coming into oestrus in 3-5 days and 56% conception within 27 days (Dobson et al, 1977). Other workers reported 80% response to treatment of PGF2 alpha with at least a 60% conception rate (Jackson, 1981). Prophylactic use of GnRH at 100-200ug at 12-14 days postpartum has been suggested to reduce the prevalence of cysts (Kesler and Garverick, 1982). However, by careful genetic selection through eliminating bulls which have sired daughters with cystic ovarian disease, its occurrence can be reduced. Unfortunately, most cows affected are the best producers.

2.2.3 LUTEAL DEFICIENCY

Luteal deficiency has been suspected of causing infertility. It was found that 50% of the nonpregnant cows which ovulated had lower plasma progesterone values six or more days after ovulation than those which conceived (Erb et al., 1976). Others have reported this phenomenon in 2% (Bulman and Lamming, 1978) and 18% of cows (Jackson, 1981). However, there were some cows that conceived even though they had this low pattern of progesterone profile. Some workers have tried to use hCG or GnRH injected after ovulation to stimulate the development and function of corpus luteum

or induce accessory CL formation (Greve and Lehn-Jensen, 1982; Sreenan and Diskin, 1983).

2.2.4 HORMONAL IMBALANCES

If there is an **incorrect balance of oestrogen and progesterone** it may influence the time of transport of the oocyte and zygote along the uterine tube, resulting in an accelerated or retarded passage of the zygote. Thus, the zygote may reach the uterus at a time when the environment is hostile to its survival. However, it has been recorded that even with elevated plasma progesterone concentrations, beef heifers failed to conceive (Corah et al, 1974). Cows infused with ACTH (Gwazdauskas et al, 1972) and subjected to high environmental temperatures (Abilay et al, 1975) also failed to conceive.

2.3 MANAGEMENT FACTORS

The role of **nutrition** has become increasingly important in recent years with the elimination of specific infectious diseases and the move towards higher production. However, the influence of nutrition takes time to become effective. At the same time, the response of the animal can be complicated by interaction of a number of factors such as present and previous body condition or level of production. Nutritional deficiencies and excesses causing infertility may act via the hypothalamus and anterior pituitary thus influencing the production of gonadotrophins or interfering with oogenesis and endocrine function. Nutrition may influence sperm transport, fertilization, early cell division and development of the embryo or foetus (Arthur et al, 1989). However, there have been other studies in cows

suggesting that hormonal concentrations in the peripheral plasma, follicular growth and fertilization rates are not affected (Spitzer et al, 1978).

There are important relationships between nutrition and reproductive function, namely at the time of puberty and between puberty and first calving. Puberty is determined by body size rather than age. At puberty, beef and dairy heifers have reached 50% and 35-45% of their mature weight respectively (Arthur et al, 1989). Heifers are normally not mated at the onset of puberty since they are not fully grown and dystocia would be common at first calving because of feto-maternal disproportion. In addition, the heifer is under considerable stress because she is still growing to physical maturity whilst maintaining a pregnancy to term. However, breeding at an early age can improve the lifetime productivity of an individual animal. For example, beef heifers which calved at two years of age rather than at three produce 0.8 more calves per cow at 10% less cost over their entire production life (Pinney et al, 1962).

The effect of inadequate nutrition, mainly deficient energy intake, is cessation of cyclical activity, silent oestrus, ovulatory defects, conception failure, and embryonic death. It has been demonstrated that cows which lost the least weight after calving and also gained weight at the time of service had a higher chance of conceiving to first service (McClure, 1961). It was reported that a 10% fall in live weight post-partum was associated with low fertility (McClure, 1970). Blood glucose values of less than 30mg/dl were found to be associated with reduced fertility (McClure, 1968).

Other management factors which are related to production and physiology of the animal would also affect reproductive efficiency, namely, methods of handling the animals, sudden change in nutrition, mishandling, design of facilities, unnecessary stress imposed on the animals amonst a variety of factors.

2.4 DISEASE

Infections affect fertility by altering the genital tract environment, resulting in impaired sperm transport and death of, or interference with the subsequent development of the conceptus. With close monitoring and eradication programmes, many of the venereally transmitted diseases such as vibriosis, trichomoniasis, and brucellosis, have assumed less importance. Conversely, diseases such as IBR/IPV, BVD/mucosal disease and leptospirosis have assumed much greater importance (Arthur et al, 1989).

Campylobacter infection causes cows to return to service irregularly, especially coinciding with the introduction of a new bull. In natural breeding it is important to use a clean bull on the herd. Brucellosis has been reduced to a very low level since 1983 in Britain. In most parts of the world bovine tuberculosis has also been eradicated. Trichomonas infection is probably non-existent in Britain (Arthur et al, 1989). Leptospirosis has long been known to occur in cattle causing fetal death, abortion, stillbirth and weak calves. Salmonellosis-induced abortion has persisted as a problem for sometime although not a major one in Britain (Arthur et al, 1989). Listeria monocytogenes, Actinomyces pyogenes and Escherichia coli are also known causes of abortion in cattle. Viral infections such as bovine virus diarrhoea, mucosal disease (BVD-MD), infectious bovine rhinotracheitis (IBR),

mycoplasmosis and fungal invasion of the placenta are known to cause embryonic death and abortion. Non-specific infections due to opportunist pathogens can cause puerperal metritis and endometritis which can also reduce fertility. Normally, infections of such a nature require a predisposing cause, such as poor calving conditions and tend to affect individual cows only.

CHAPTER THREE

3.0 MATERIALS AND METHODS

Two herds of British Highland Cattle in two different locations were used in this study. The first herd was in Glasgow (Farm 1) and the second herd was in Oban, Argyll (Farm 2). The basis of selecting these two farms for investigations was because of the earlier involvements of Dr. M.J.A. Harvey and Dr. L. Robertson from the Veterinary Faculty of the University of Glasgow, Scotland in the infertility problems in farm 1 and farm 2 respectively. The scenerio of infertility in farm 1 constituted an increased rate of abortion during the third trimester and the number of prolonged service intervals when apparently pregnant animals were found to be opened near term. No organism was identified in the abortion cases carried out by the Government research laboratory. In farm 2, the infertility syndrome involved a group of heifers in which the animals failed to conceive despite being mated repeatedly by the bull. The heifers showed normal oestrous cyclicity. Preliminary results had demonstrated adhesions in the ovarian bursa and cervical strictures. Thus, it became apparent that a detailed study into the problems of infertility in the British Highland Cattle on these two farms was required to elucidate some of the causes of infertility in the herds. Further investigations were then conducted in farm 1 on chromosomal analysis, monitoring of pregnancy and post partum ovarian cyclicity. In farm 2, further chromosomal investigations and post-mortem studies on reproductive tracts of the infertile cows and heifers were carried out.

3.1 FARM MANAGEMENT

An infertility investigation was conducted upon request on farm 1, which experienced a drop in fertility shown by prolonged calving interval and the low number of calves born. Farm number 1 belonged to the city of Glasgow and was situated about 4 miles away from the city centre. This farm had an acreage of 72.9 hectares with a total of 50 breeding head of British Highland cows whose ages range from 3-18 years. The pasture was fertilised twice a year at a rate of 420kg/hectare of N:P:K at a ratio of 20:10:10.

Farm 2 was a private farm situated about 70 miles from Glasgow in Oban. It had a herd of about 50 head of BHC and about 1,200 head of Blackface cross ewes. In both of these farms, the cows were managed on a semi-intensive free range system and housing was not provided throughout the four seasons. Rotational grazing was practiced. The animals were fed mainly on grass and hay.

3.2 FARM 1

Concentrate feeding was given as a supplement 6 weeks before calving from December to April or whenever there was a drop in quality of pasture. Yearlings and heifers were not fed with concentrate, but only with hay during this period. Mineral was provided only in the concentrate. Water was provided in the water trough and from the surrounding rivers.

This herd utilized natural service, using four bulls and heifers were bred for replacement in a closed herd. New bulls were occasionally bought for mating in the farm to avoid inbreeding. The animals were occasionally tested for IBR, annually tested for Enzootic Bovine

Leucosis and once every 2 years for Tuberculosis and Brucellosis. Variable time was spend on oestrous detection during the period of study which was carried out on an ad hoc basis wherever possible. After calving in December 1991, the bulls were introduced into the herd for about 5 months from March to July, 1992. Bulls were allowed to run with the cows for a period of 2 months each on a rotational basis. The calves were allowed to suckle ad libitum and were weaned from the dams at 9 months of age.

3.2A DATA SOURCE AND INDICES

Breeding records consisting of calving dates from 1986 to 1991 of the herd were analyzed using a commercial computer spreadsheet (Excel ,Microsoft, USA). The herd data were entered into a worksheet consisting of empty cells. Data entry consisted of calving dates, service dates, progesterone values, pregnancy diagnosis, rectal palpation and chromosomal findings. Basic calculations were carried out by using formulas to produce meaningful values from the data. Thus, days of calving to conception, calving to first service, inter-service interval, plus the current postpartum and post-service stage could then be obtained. These data were easily updated by inserting them into the cells as the worksheet had 256 columns and 16,384 rows. This meant that the worksheet can contain at least 4 million data. Thus, all the cows in the herd can be recorded into a single spreadsheet used for data analysis, decision making, calculations and reporting. These breeding records would provide the various reproductive indices to measure the level and trend of fertility of the herd. The pedigrees of the selected cows were traced for five generations using herd record books from the British Highland Cattle Society. This was to determine

the source and pattern of inheritance of the chromosomal defect.

i CALVING INDEX

This was the average calving interval between the last two calvings of a group of cows measured in days. The formula used was :-

Total calving intervals of the herd/ Total number of animals

ii CALVING INTERVAL

This was the interval from one calving to the next for an individual cow. The calving interval consisted of four parts namely the interval between calving date and the earliest oestrous date, the interval between the latter and the first service date, the interval between that and the date of conception and interval from conception to calving (ANON, 1984). Thus any of these factors can affect the calving interval. The earliest service date post-partum would depend on management decision. The average calving interval became the calving index as described above.

iii OESTROUS DETECTION

Oestrous detection referred to the percentage of oestrous periods observed in relation to the number of oestrous periods that were assumed to have occurred.

iv INTER-OESTROUS INTERVAL

The level of accuracy of oestrous detection can be obtained by examination of the frequency of the inter-oestrous interval divided into the following groups:

2-17 days, 18-24 days, 25-35 days, 36-48 days or more than 48 days.

v CALVING TO CONCEPTION INTERVAL

This was one of the components of the calving interval. It could be calculated retrospectively by deducting the gestation period (280) days from the calving interval. Alternatively, it was the interval in days from calving to the service which resulted in pregnancy. The formula used was :-

The sum of the calving to conception intervals/ The number of cows which conceived

vi CONCEPTION TO 1ST SERVICE

This was the number of first services which resulted in pregnancy expressed as a percentage of the number of first services. In this instance, conception was diagnosed at 35 days. The formula used was :-

Number of cows pregnant to first service X 100 / Number of cows receiving first service

vii PREGNANCY RATE TO ALL SERVICES

This was the number of cows diagnosed pregnant expressed as a percentage of the total number of services.

Number of cows pregnant X 100 / Total number of services

viii PERCENTAGE PREGNANT OF COWS SERVED

This was the number of cows diagnosed pregnant expressed as a percentage of the total number of cows served.

ix SERVICES PER CONCEPTION

It was the number of services divided by the number of pregnant cows as shown by the formula.

Total number of services X 100 / Total number of cows pregnant

x CU-SUM OF PREGNANCY RATE

This was a useful method of monitoring the contemporary fertility trend of the herd by recording the pregnancy rates to all services or first services in chronological order.

xi FERTILITY FACTOR

This was another means of measurement of fertility as described by Esselmont et al (1985). It was derived from this formula.

Oestrous detection rate X Overall pregnancy rate / 100

3.2B CLINICAL EXAMINATION

Determination of ovarian structures and function is essential in evaluating the potential of the cow to produce normally. The state of ovarian function can be assessed both directly or indirectly by the presence of CL or ultrasonography and progesterone concentration

respectively. However, the combined use of clinical examination, progesterone concentration and ultrasonography were employed at random to enhance the accuracy of the assessment of the events of the reproductive tracts and ovarian function. The clinical examination of the reproductive tracts of the cows was conducted once a week for a period of 3 months from 10th March to 9th June 1992 and thereafter at irregular intervals whenever required. Routine examinations were carried out by rectal palpation of the reproductive organs with special emphasis on the ovarian functions. Recognition of CL by rectal palpation can be used to differentiate cycling and non-cycling cows (Zemjanis et al, 1969). Ultrasonic examination and progesterone assays were used to aid in the investigations. The animals presented for examination were mainly cows of more than 30 days post-partum, cows not showing oestrus after more than 42 days post-partum and cows that had been mated 30 days or more before by the bulls.

3.2C PROGESTERONE ASSAY

Blood samples were taken from the tail vein of the cow into sterile tubes containing lithium heparin (Monovet,Sarstedt) as the anticoagulant. The blood samples were taken for the purpose of chromosomal analysis and analysis of progesterone concentration. Once weekly progesterone levels were taken to assist in the clinical examination of infertility. However, only random samples were taken as required or when the situation warranted. Those blood samples for progesterone assay were then centrifuged at 150 g for ten minutes. The plasma was removed and stored in plastic tubes in the freezer at -20^{o}C until analysed.

The levels of circulating progesterone were determined using the ELISA kit (Ovucheck, Cambridge Veterinary

Science. Ltd). The test was based on the competitive binding of unlabelled progesterone present and a fixed quantity of progesterone labelled with the enzyme alkaline phosphatase (AP) to the binding sites on a limited amount of specific progesterone antibody. The wells were precoated with antibody providing a solid phase. After incubation, all components except those bound to the plate wells were washed away. The amount of bound AP-labelled progesterone remaining on the wells was inversely proportional to the concentration of the unlabelled progesterone present in the sample. During the second incubation, the bound labelled progesterone was then measured by reacting the AP with its substrate. A spectrometer was used to measure the colour produced and the concentration was determined from a standard curve.

3.2D ULTRA-SONIC SCANNER

A 'real time' B-mode ultrasound scanner (Concept II Dynamic Imaging, Livingstone) equipped with a 7.5MHz linear array transducer which produced a picture of the contents of the uterus and ovarian structures was used. The technique employed was transrectal ultrasound examination as described by Boyd et al, (1988). The findings helped to substantiate and to confirm or disprove the rectal findings when required. In ultrasonography, the follicles appeared non-echogenic whereas the CL appeared echogenic.

3.3 FARM 2

3.3A ABATTOIR SPECIMENS OF REPRODUCTIVE TRACTS

Reproductive organs of infertile British Highland cattle from farm 2 consisting of 8 cows and 4 heifers slaughtered at the Paisley abattoir were collected for

the study. These tracts were examined macroscopically for any gross abnormalities and photographed. Cervical patency was checked by introducing an AI pistollet through the cervix.

Uterine body swabs were taken for bacterial culture. These were done by sterilising the external uterine body with a hot scalpel blade before the sterile swabs were introduced in order to avoid any contamination. The uterine lumen was swabbed several times before withdrawing. The swabs were then cultured aerobically on sheep blood agar and Maconkey agar.

Uterine tissue sections were processed for histological studies. These were done by removing uterine tissue samples of about 2cm square and fixing them in Bouin's fixative for at least 24 hours at room temperature before being processed. The process was carried out using an automatic Histokine 24 hour tissue processor (Shandon Elliot, Ltd). The specimens were dehydrated through a series of upgraded alcohols containing 5% phenol to keep the tissue soft. The tissues were then impregnated with 1% colloidin and methyl benzoate, cleared in xylene, impregnated with two changes of paraffin wax and finally blocked out in fresh wax. 5u sections were cut from the blocks using an optical Spencer 821 microtome. These sections were mounted on glass slides and dried in an oven at 56^{o}C for an hour. They were stained in haematoxylin and eosin prepared according to Culling (1974). Histopathological examinations were carried out using a Leitz Orthomat microscope with 10x eyepiece and x100 objective.

The oviducts were tested for patency by using the method described by Kelly (1981) with minor modifications. The tip of the uterine horn was occluded by clamping the uterine horn between the index finger

and the thumb. About 10ml of phenolsulphonphthalein (PSP) dye was aspirated into a 10ml syringe. The dye was injected into the horn using an 18g needle above the occlusion held by the fingers. The dye can be seen to pass along the oviduct and, if patent, to flow out from the fimbria. Conversely, the dye will be dammed up along the oviduct without passing out from the fimbria if it was non-patent. The same process was repeated for the other horn.

3.4 FARM 1 AND FARM 2

i CYTOGENETIC INVESTIGATION

A total of 68 blood samples were obtained from the two different herds of British Highland cattle. When any animal was identified as a carrier of the Robertsonian translocation, as many offspring and relatives as possible were analyzed for the chromosomal aberration. The analysis carried out was by the conventional culture technique (Moorhead et al, 1960) and the translocation was identified by using G- and C-banding techniques described by Wilson (1988).

10ml of heparinised blood was collected from the caudal vein in a sterile lithium heparin tube (Monovet, Sarstedt). 2ml aliquots of blood were transferred into 10ml sterile plastic tubes (Greiner, Laborteknik) containing 9ml of culture medium in a laminar flow cabinet (Pathfinder). The culture medium used was RPMI 1640 (Flow Laboratories, Irvine) supplemented with 20% foetal calf serum, 75iu/ml penicillin, 75ug/ml streptomycin, 60ul 200M L-glutamine (Flow Laboratories) and 1.875mg phytohaemagglutinin (Wellcome, Beckenham). The tubes were cultured at 37^{o}C for 46 hours. At the end of incubation 0.1ml of 10ug/ml colcemid solution

(Sigma) was added. Further incubation for 1.5 hour was carried out after the treatment of colcemid. On completion of incubation, the blood cell culture was centrifuged at 150 relative centrifugal force (g) for 10 minutes. The supernatant was aspirated by vacuum suction using a negative water pressure leaving about 1ml of cells. The cells were resuspended in 10ml of prewarmed hypotonic solution (0.022M Potassium chloride). The cells were dispersed by vigorous pipetting using a pipette. This suspension was then incubated at 37^{o}C for 15 minutes.

After the hypotonic treatment, the cells were centrifuged and supernatant removed in the same manner as described above, leaving only a small button of cells of about 0.1ml. Fixation of the cells was carried out twice using freshly prepared cold fixative (methanol : glacial acetic acid at 3:1). This was done by adding 5ml of the fixative into the small button of cells and vigorous pipetting to disperse the cells. The resuspended cells were refrigerated at 4^{o}C for 15 minutes before the tubes were recentrifuged at 150 g for 5 minutes. The supernatant was discarded and a second fixing process was carried out by adding 2ml of fresh fixative to the button of cells. Resuspension of the cells by vigorous pipetting was done and they were then refrigerated for at least 30 minutes before replacing the fixative. The cells were centrifuged again at 150 g for 5 minutes and the supernatant discarded as earlier described. The remainder of the cells were finally resuspended in 0.5ml of fixative by pipetting and dropped onto the microscopic slides, held at an angle of 45 degrees. The slides were allowed to dry, stained with freshly prepared 10% giemsa stain (Gurr's R66 in buffer pH6.5, BDH Chemicals) and fixed with a coverslip. The prepared slides were examined under a light microscope (Leitz, Ortholux) at 1000X

magnification for translocations. This was done by counting the number of chromosomes and observed for structural abnormality.

ii G-BANDING AND C-BANDING

Chromosome preparations which were at least seven days old were used for G-banding. These chromosome preparations were examined under a phase contrast microscope (Vickers, Photoplan) for the selection of metaphase spreads. The method of staining used was as described by Wilson (1988).

Stock trypsin 2.5% (Flow Laboratories) was stored in 0.6ml aliquots at -20^{o}C until use. The concentration of freshly prepared trypsin used was 0.03% prepared by diluting 0.6ml aliquot of 2.5% trypsin with 50ml of Hank's Balanced Salt Solution. The slides containing fixed chromosome preparation were immersed in this trypsin at room temperature (18-27^{o}C) for 4-15 minutes depending on the extend of trypsin digestion of the chromosome which can be observed under phase contrast microscopy. The slides were dehydrated through 70, 95 and 100% alcohol and rinsed in deionised water and air dried. They were then stained for 3 minutes in 1.5ml Giemsa (Gurr R66, B.D.H. Chemicals) diluted with 40ml of Sorensen's buffer of pH 6.8. The stained slides were dipped in xylene and mounted in D.P.X. (B.D.H. Chemicals) with a coverslip.

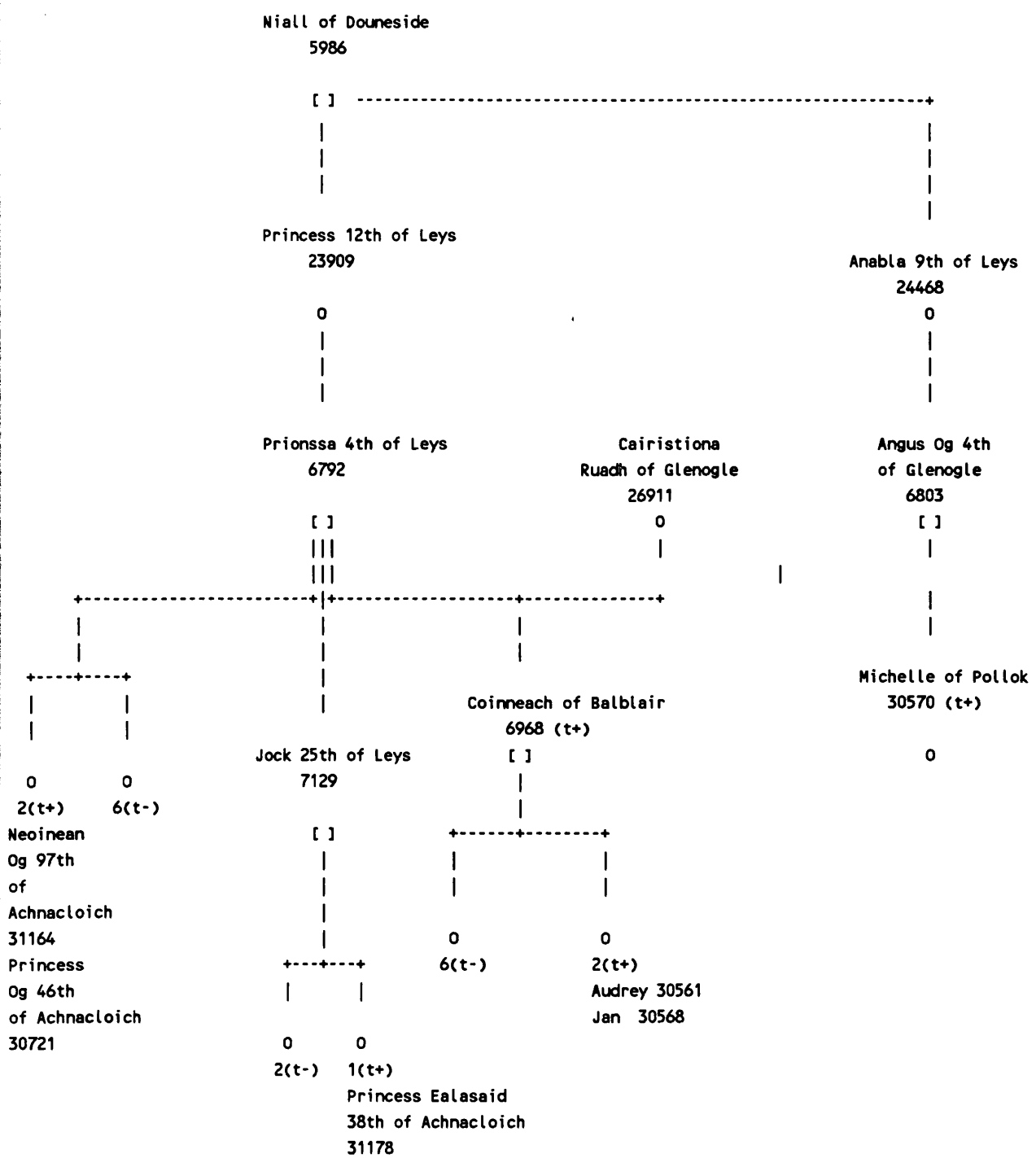
The technique of C-banding was carried out as described by Sumner (1972). The slides containing fixed chromosome preparations were treated with 0.2N hydrochloric acid for one hour at room temperature (18-27^{o}C). A fresh solution of 5% barium hydroxide was prepared by boiling 5g barium hydroxide (octahydrate) in 100ml distilled water. This supersaturated solution

was filtered (Number 1, Whatman) to remove undissolved crystals. The layer of scum was removed by using a tissue paper. The slides were removed from the acid solution, rinsed in deionised water and immersed in the barium hydroxide solution for 5-15 minutes at 50°C. The slides were incubated for one hour at 60°C in 2X SSC (0.3M Sodium chloride containing 0.03 M trisodium citrate), rinsed in deionised water and stained for one and the half hour with 1:50 Giemsa solution (Gurr's R66: buffer pH 6.5, B.D.H. Chemicals). The slides were again rinsed with deionised water, dried, dipped in xylene and mounted in D.P.X. (B.D.H. Chemicals) with a coverslip. Examination for the G and C banding were carried out under microscope at X1000 magnification.

iii PEDIGREE STUDY

Pedigree studies provided the genetical background to the identification of the source of the inheritance of the defect. An examination of the probands' pedigree for 5 generations was carried out from the registered pedigree book of Highland Cattle Society of Great Britain and the hypothesis of inheritance was postulated as shown in Fig 1-9.

FIG 1 HYPOTHESIS OF INHERITANCE OF TRANSLOCATION



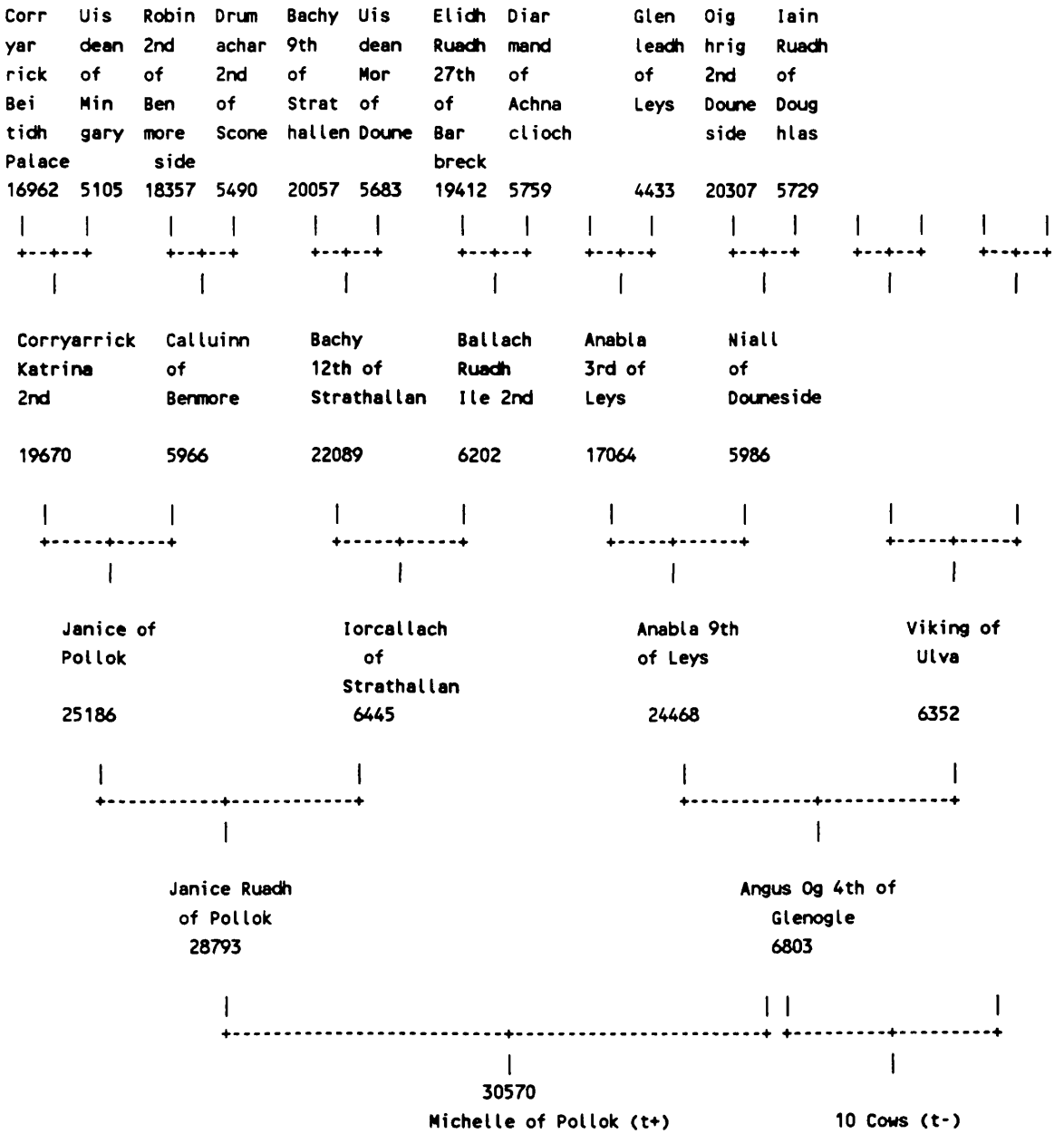


FIG 2 PEDIGREE OF MICHELLE OF POLLOK

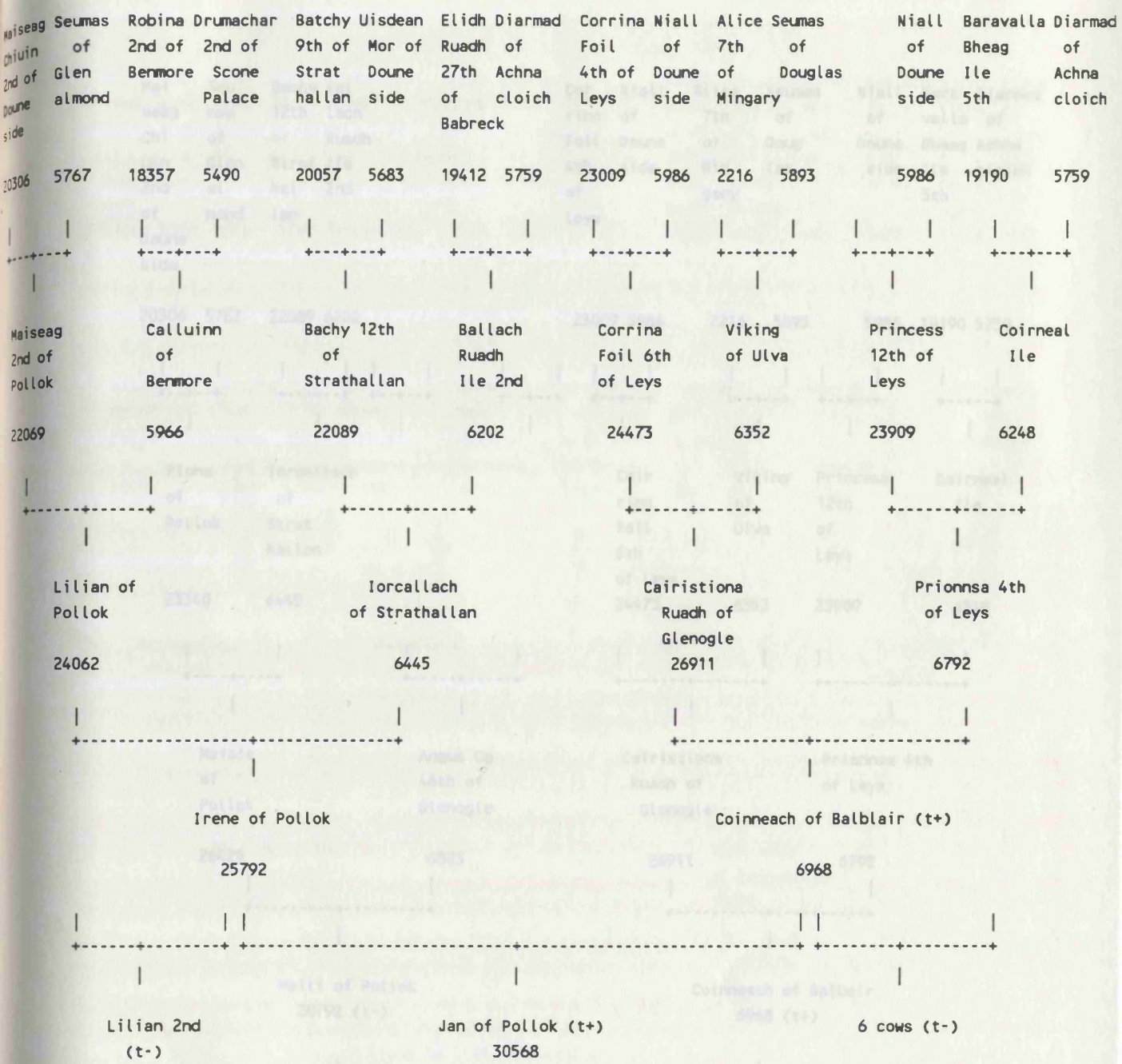


FIG 3 PEDIGREE OF JAN OF POLLOK

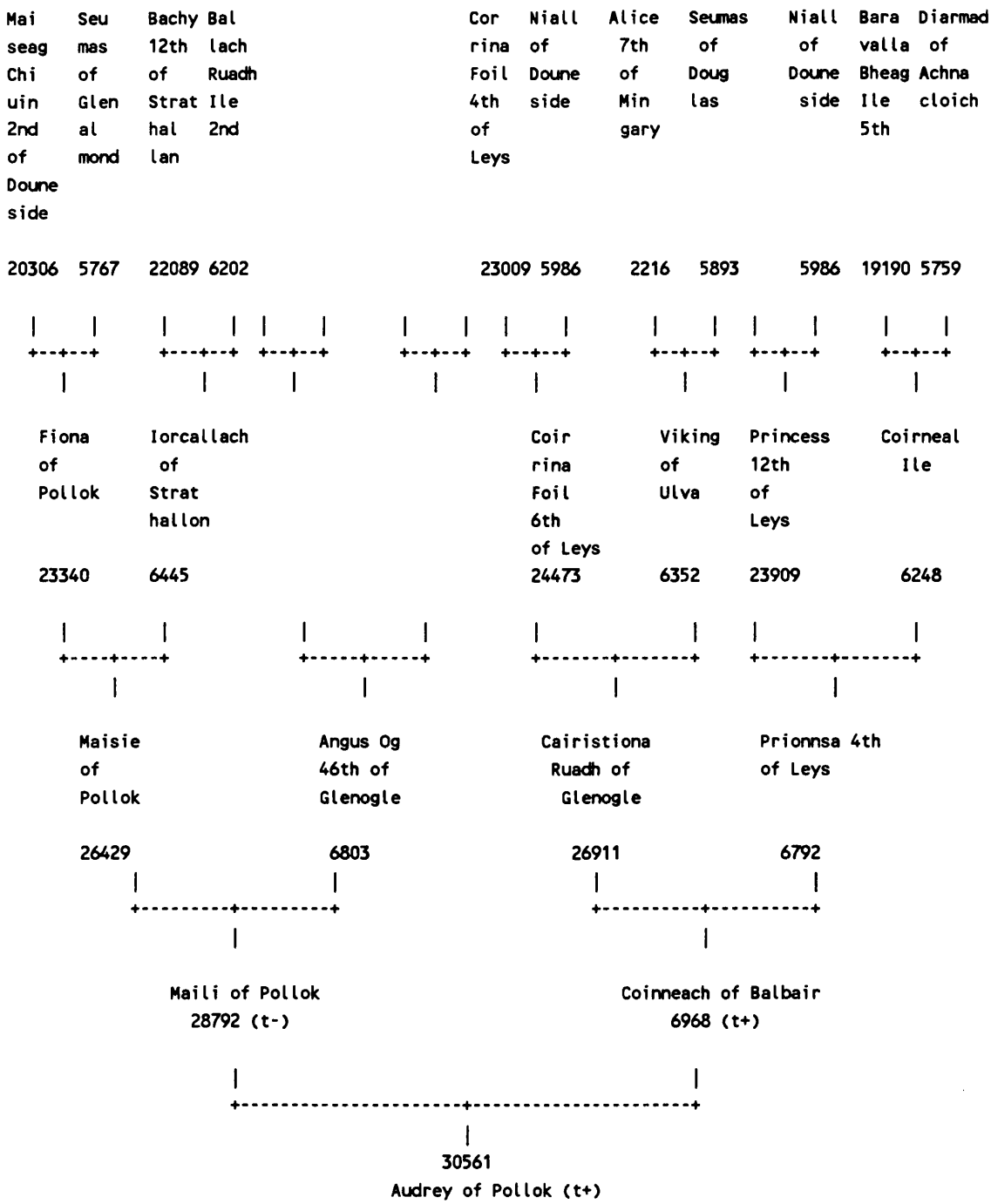


FIG 4 PEDIGREE OF AUDREY OF POLLOK

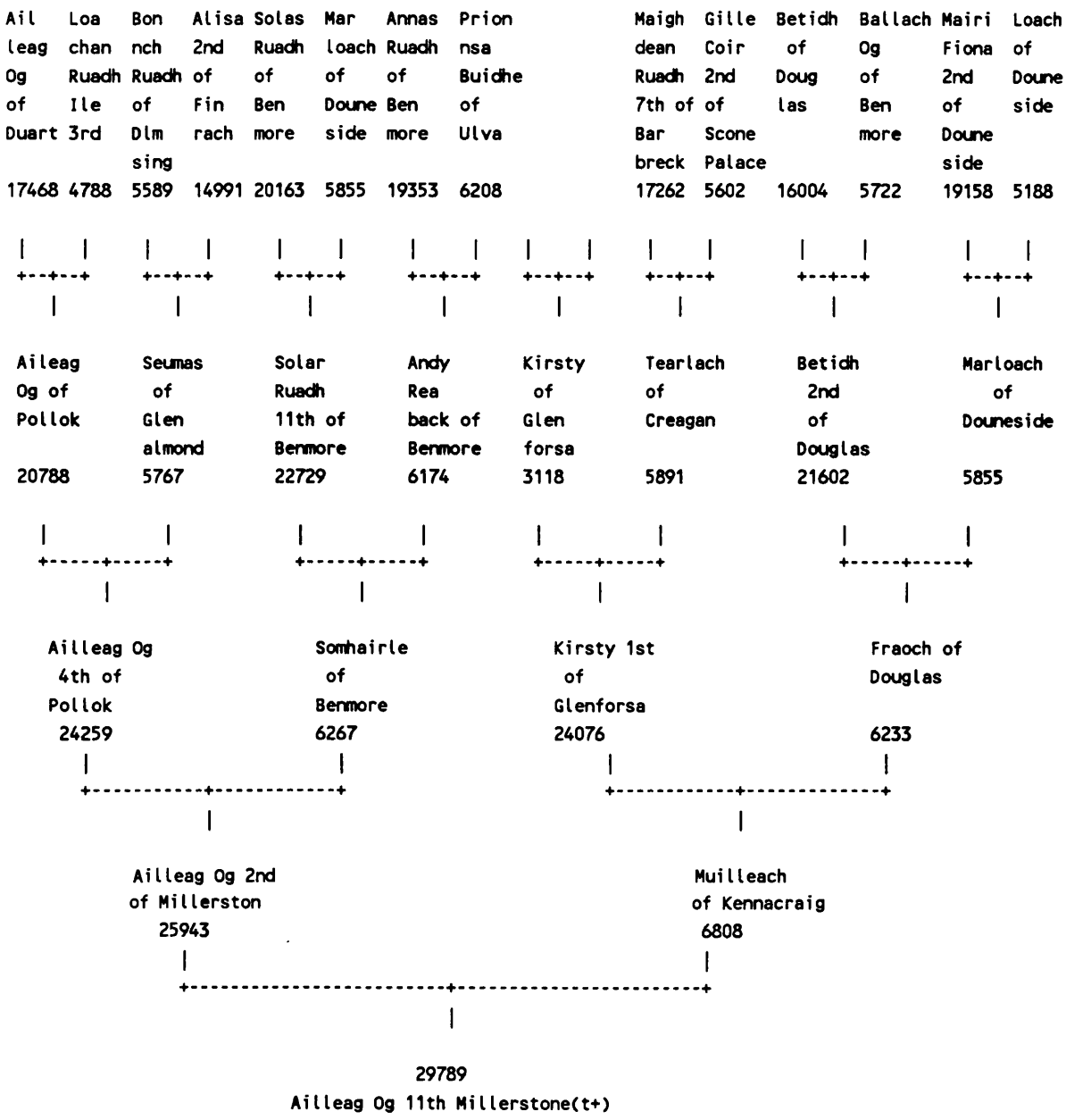
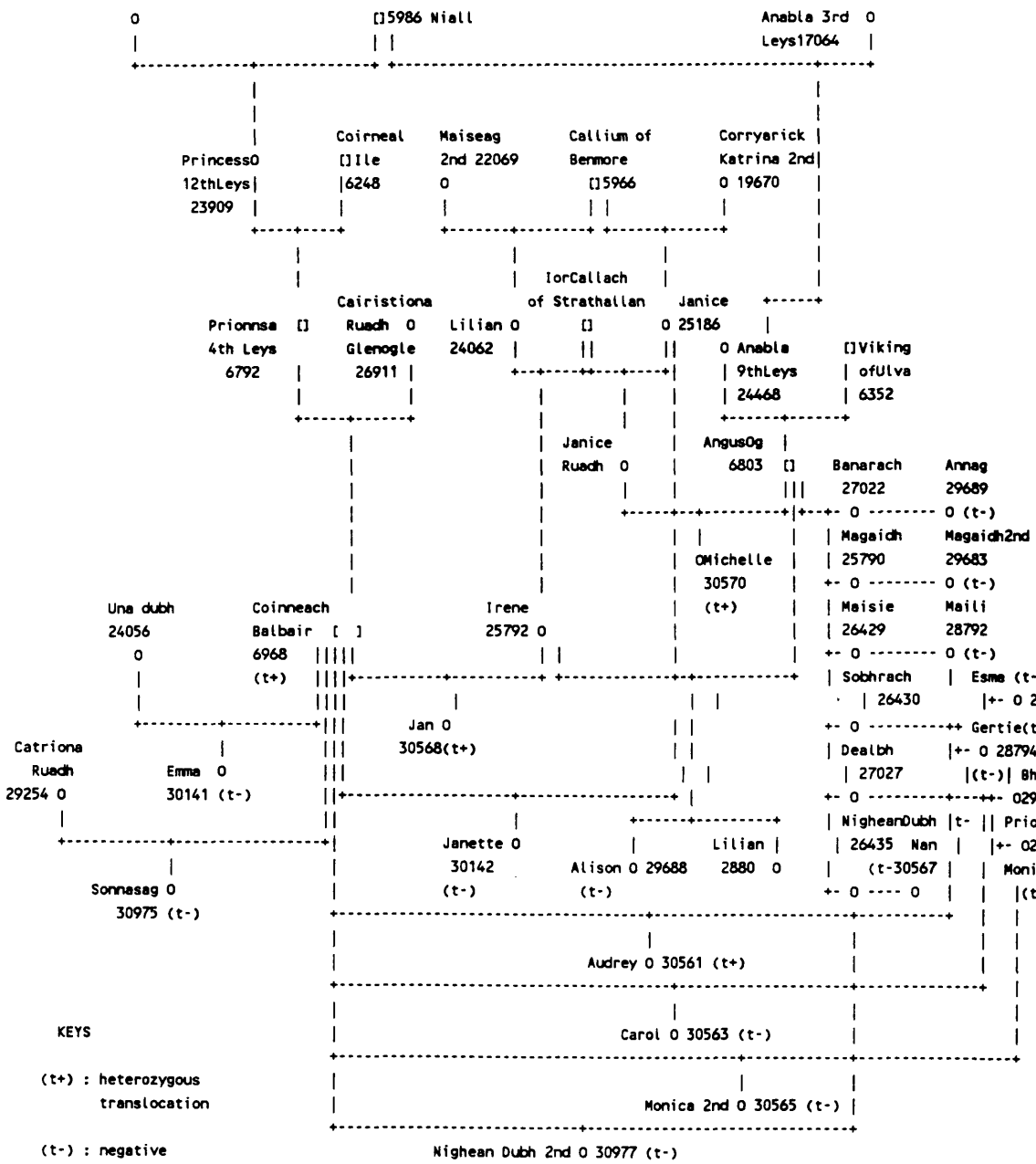


FIG 5 PEDIGREE OF AILLEAG OG 11TH MILLERSTONE

FIG 6 PEDIGREE OF PROBANDS IN POLLOK FARM



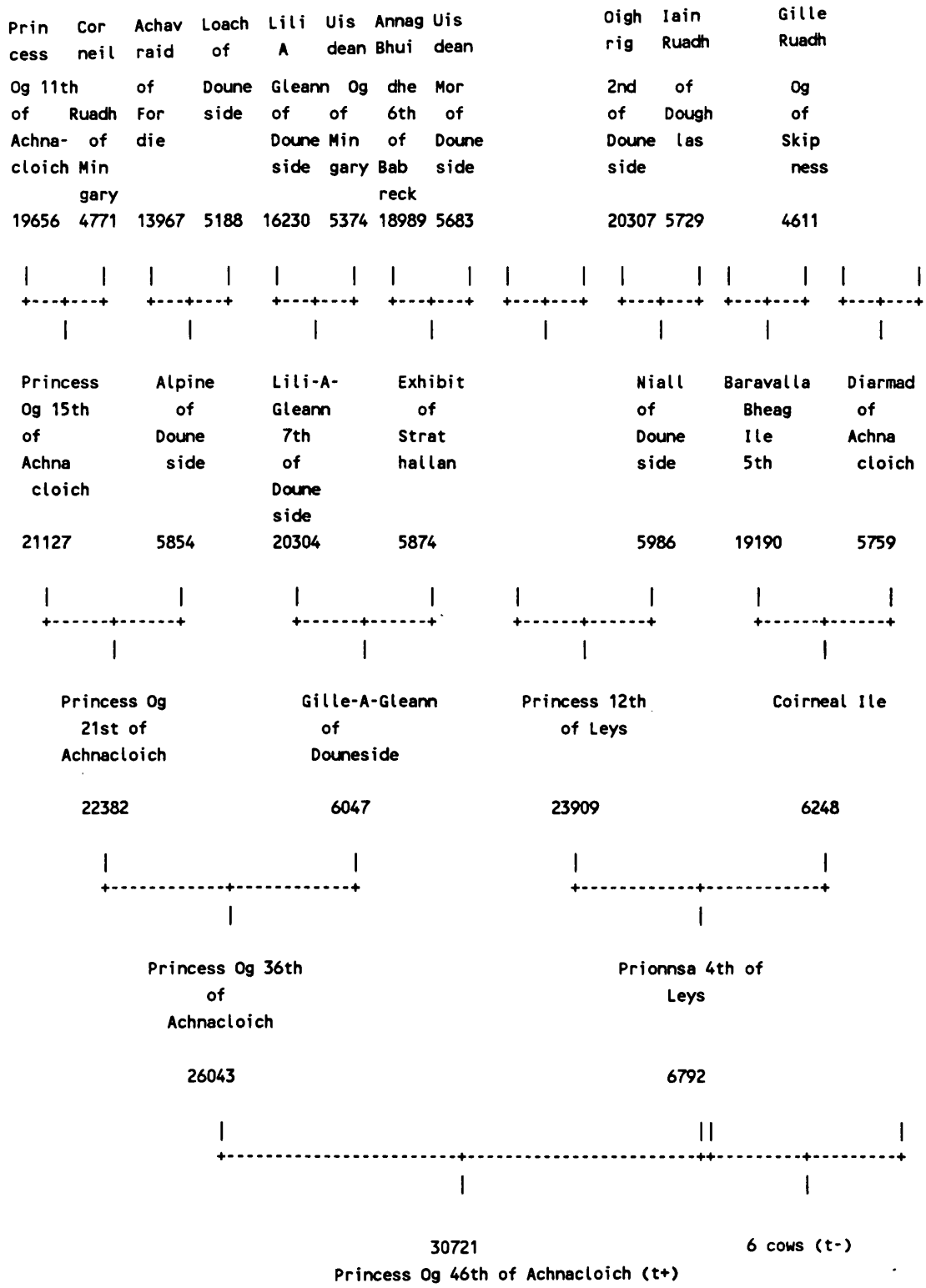


FIG 7 PEDIGREE OF PRINCESS OG 46TH OF ACHNACLOICH

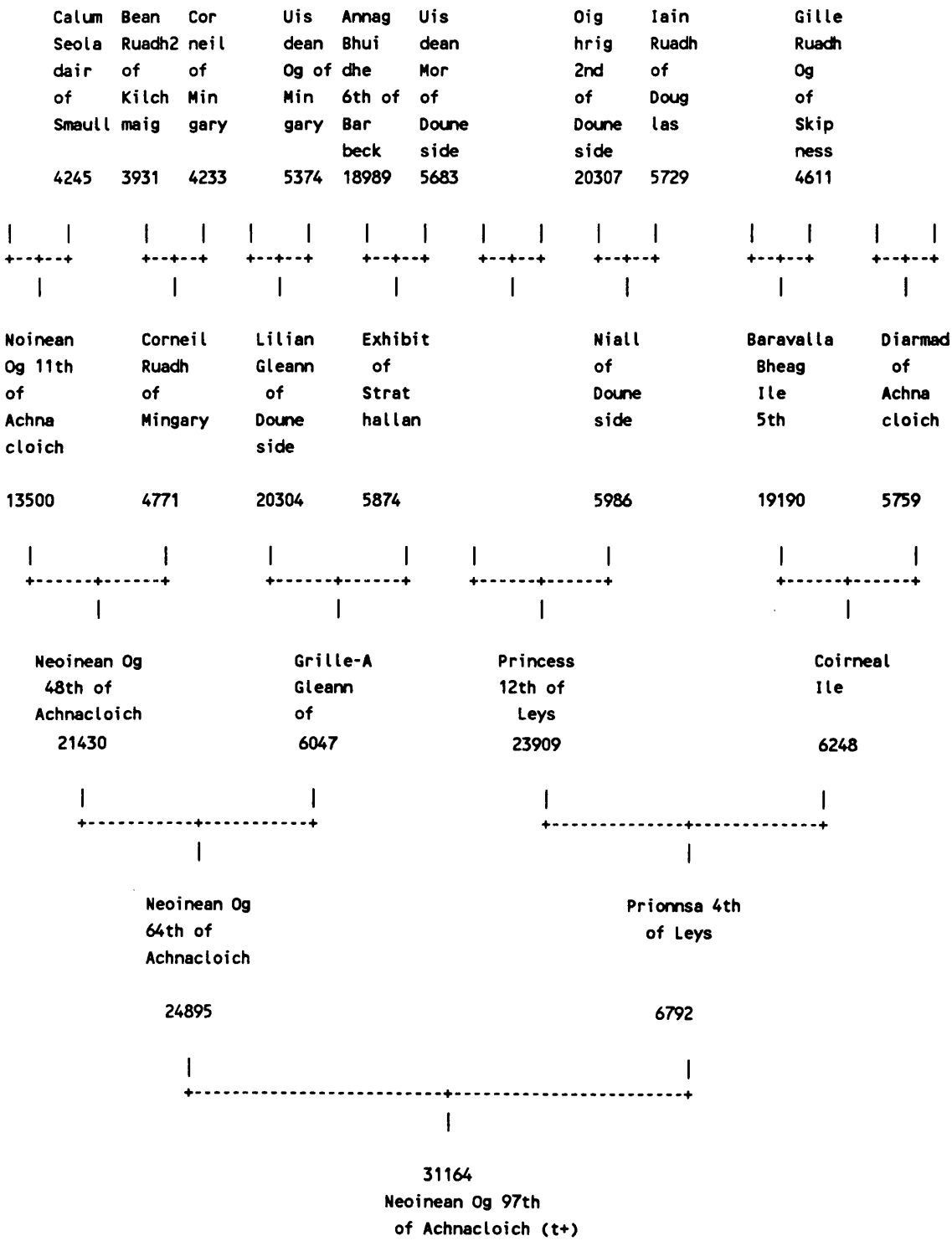


Fig 8 PEDIGREE OF NEOINEAN OG 97TH OF ACHNACLOICH

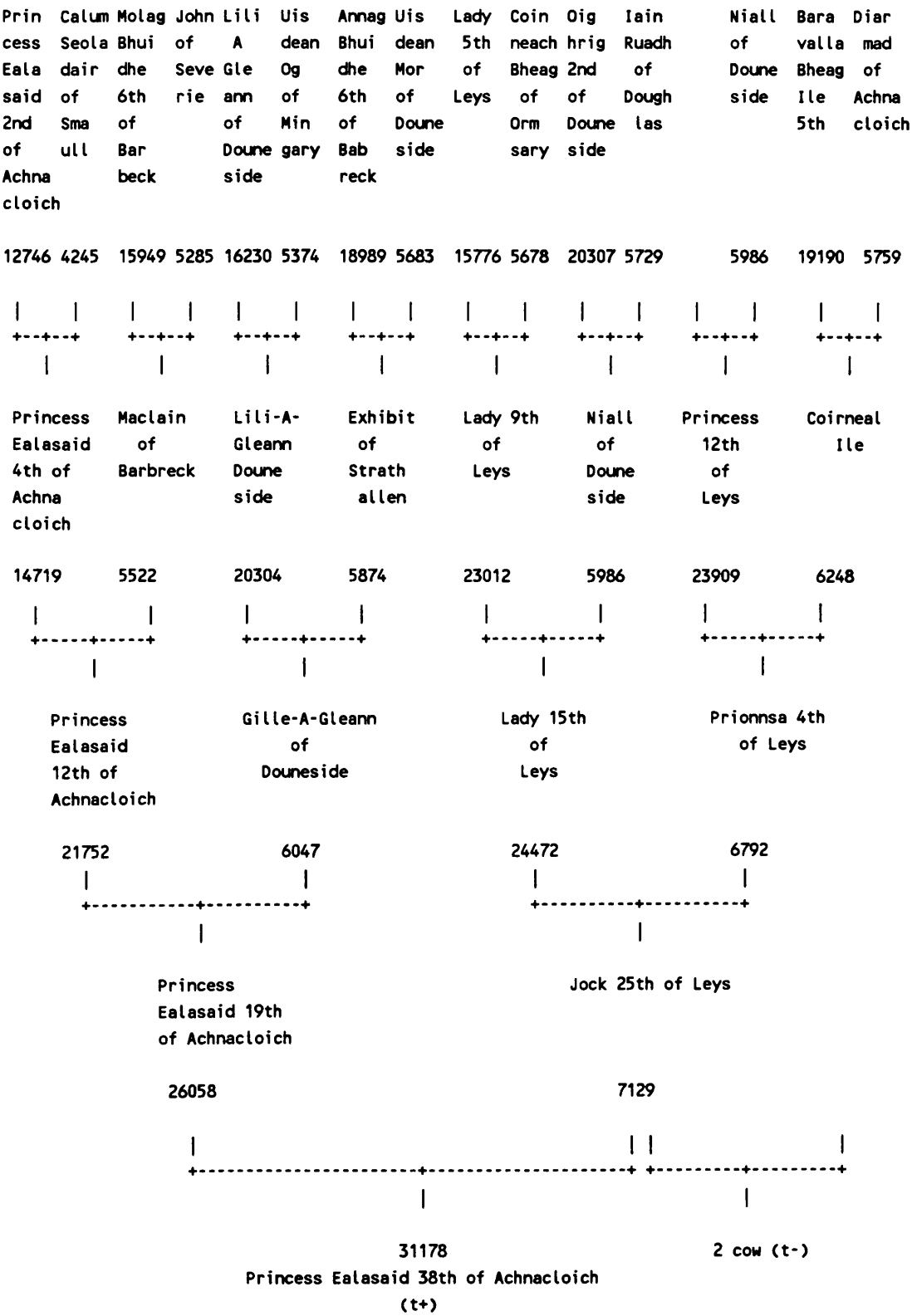


FIG 9 PEDIGREE OF PRINCESS EALASAID 38TH OF ACHNACLOICH

CHAPTER FOUR

4.0 RESULTS

There are many ways of measuring herd fertility although some are more useful than others. However, no single measure gives a complete picture of the overall level of fertility. Therefore, all the measures available were incorporated and interpreted accordingly. The following were the results of the analysis of the reproductive records using the indices described earlier in para (3.2).

4.1 FARM 1

i CALVING PATTERN

These calving patterns (Table 1; Graphs 1, 2) provided information on the mating season of the cows. Most of the calving were concentrated within the three months from March to May (spring calvings) with the mating season occurring between June and August.

ii CALVING INDEX

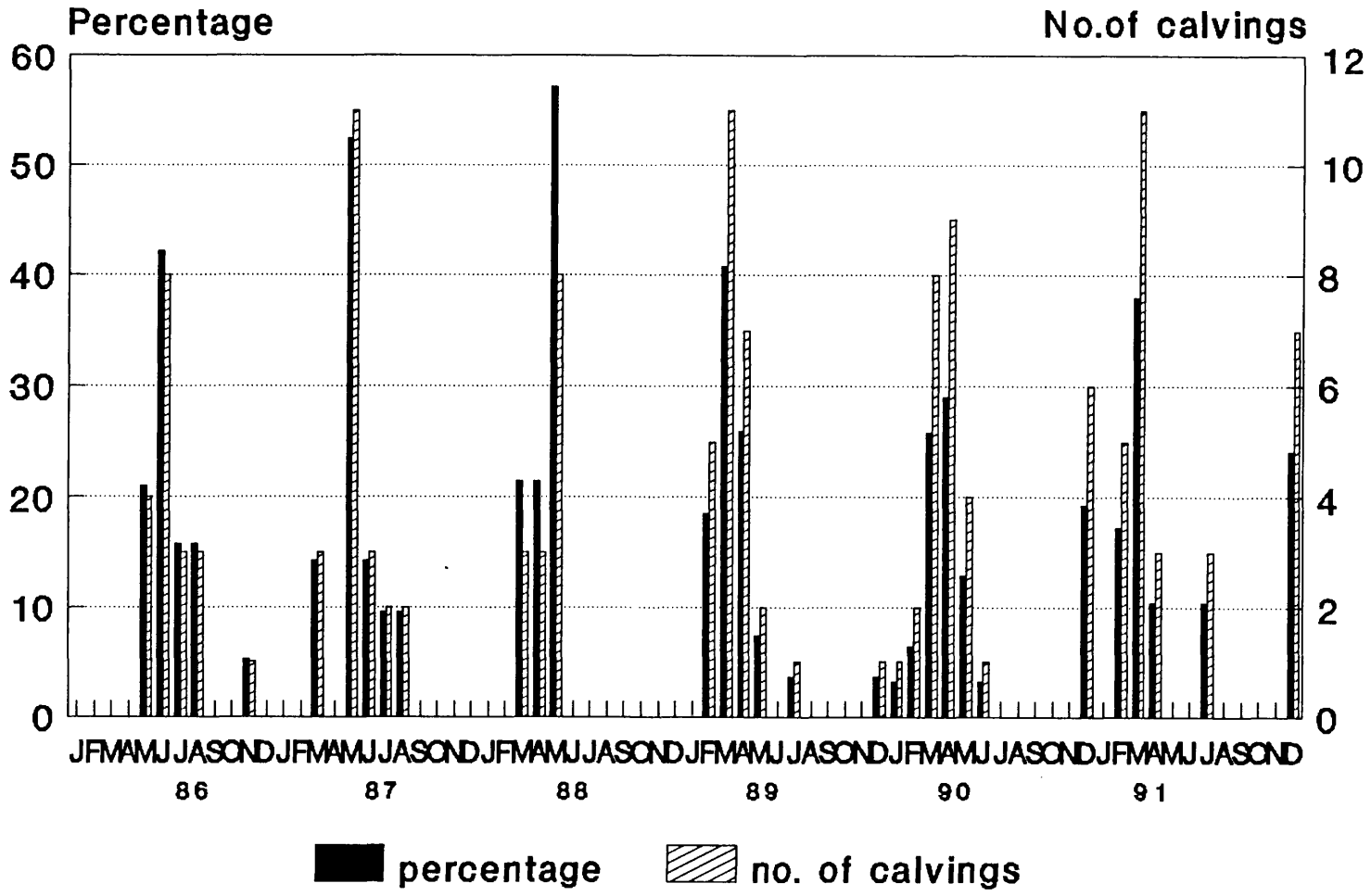
The calving indices for the various years 1987, 1988, 1989, 1990, 1991, 1992 were 364±30, 358±24, 476±181, 388±60, 392±103 and 359±34 days respectively. The average calving index was 389±45 days (Table 2; Graph 3). There was a significant difference at p=0.002 between the calving index of 1989 and the calving indices of the other 5 years using ANOVA test. However, there was no significant difference at p=0.3 between the years excluding 1989 when tested by ANOVA. This indicated that the calving index in 1989 was significantly higher than the rest of the years.

TABLE 1 : MONTHLY CALVINGS OF BHC AT POLLOK FARM
1986-1991

MONTH	YEAR													
	1986	%	1987	%	1988	%	1989	%	1990	%	1991	%	TOTAL	%
JAN	0	0	0	0	0	0	0	0	1	3.23	0	0	1	0.71
FEB	0	0	0	0	0	0	5	18.52	2	6.45	5	17.24	12	8.51
MAR	0	0	3	14.29	3	21.43	11	40.74	8	25.81	11	37.93	36	25.53
APR	0	0	0	0	3	21.43	7	25.93	9	29.03	3	10.35	22	15.60
MAY	4	21.05	11	52.38	8	57.14	2	7.41	4	12.90	0	0	29	20.57
JUN	8	42.11	3	14.29	0	0	0	0	1	3.23	0	0	12	8.51
JUL	3	15.79	2	9.52	0	0	1	3.70	0	0	3	10.35	9	6.38
AUG	3	15.79	2	9.52	0	0	0	0	0	0	0	0	5	3.55
SEPT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OCT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NOV	1	5.26	0	0	0	0	0	0	0	0	0	0	1	0.71
DEC	0	0	0	0	0	0	1	3.70	6	19.35	7	24.13	14	9.93
TOTAL	19	100	21	100	14	100	27	100	31	100	29	100	141	100

GRAPH 1 YEARLY CALVING PATTERN

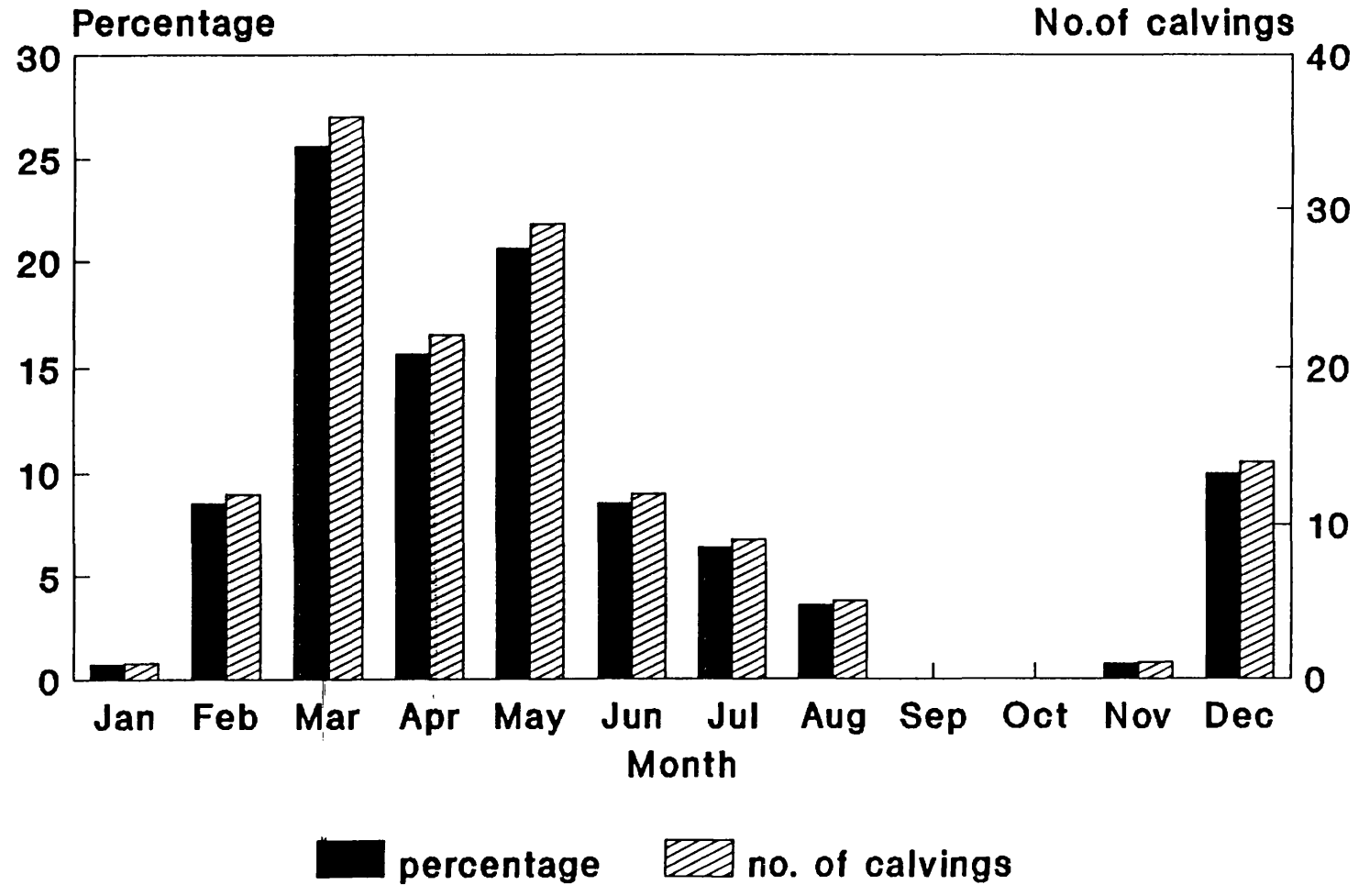
1986-1991



Total of 141 calvings in Pollok farm

GRAPH 2 AVERAGE CALVING PATTERN

1986-1991



Total of 141 calvings in Pollok farm

TABLE 2 HERD FERTILITY 1986-1991

i.	Average calving index	386.76 days
	Standard deviation	47.62
	(Total of 138 calvings)	
ii.	Calving to conception	79.20 days
	Standard deviation	34.29
	(Total of 20 cows)	
iii.	Average abortion per year	7.73 (7/54)
	Standard deviation	6.37
iv.	Average culling rate	16.97
	Standard deviation	9.28
v.	Average calf mortality	3.43
	Standard deviation	2.62

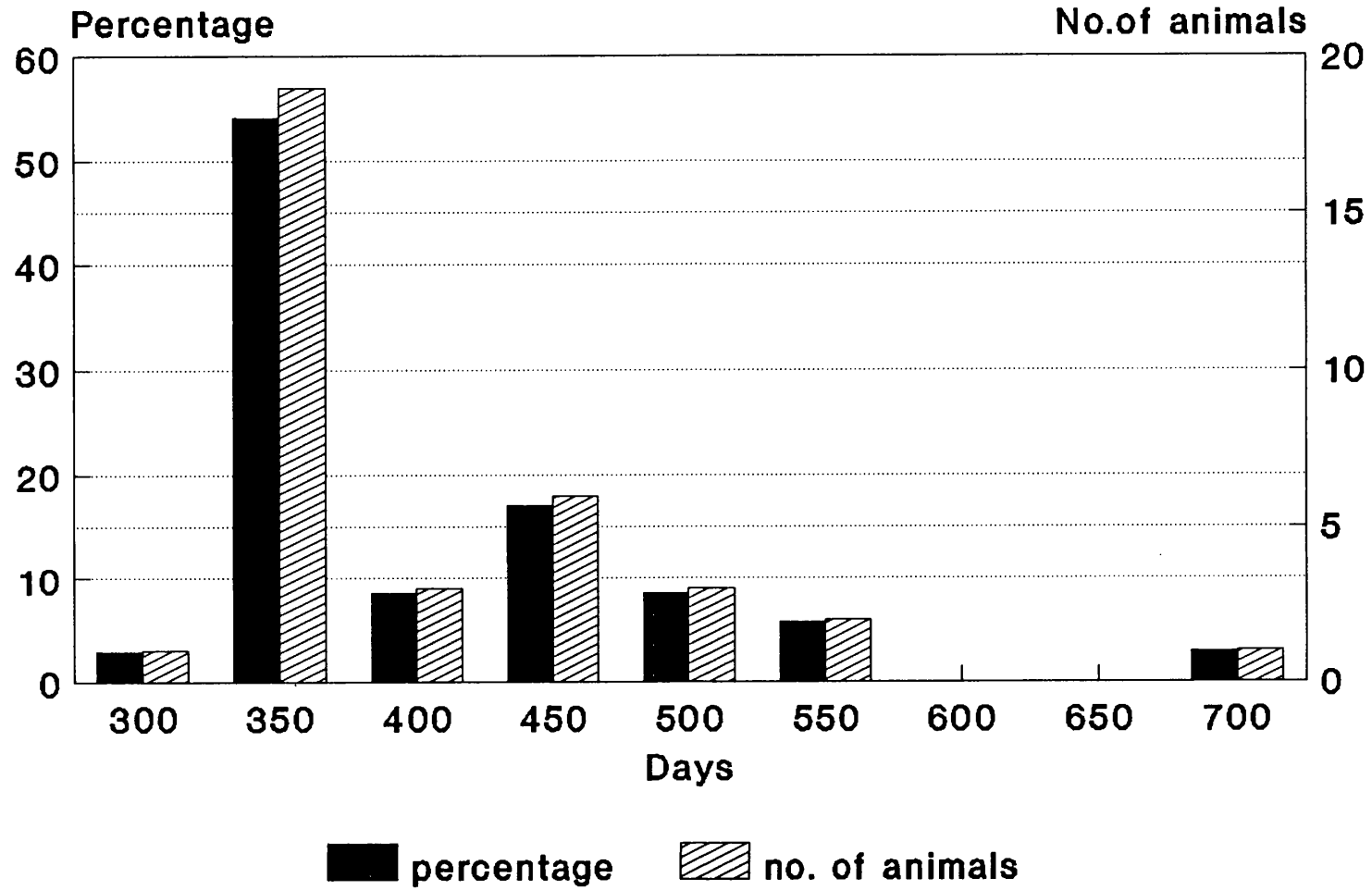
Assumptions:

i. All calving intervals were considered regardless of management decision or policy to delay breeding.

ii. The calving to conception interval is calculated by deducting 280 days from the calving interval .

GRAPH 3 AVERAGE CALVING INTERVAL

1986-1991



Total of 103 calvings in Pollok farm

iii OESTROUS DETECTION

The oestrous detection rate was 44% for the current year (Table 1). However, the accuracy of this result was based on the assumption that cows were in oestrus from clinical and progesterone values even if there were no signs of oestrous detected.

iv INTER-OESTROUS INTERVAL

They were found to be 5.88%, 64.71%, 17.65%, 5.88% and 5.88% for the various range of intervals selected (Table 3).

v CALVING TO FIRST OESTROUS INTERVAL

This was difficult to derive as the majority of these animals were more than 60 days after calving which meant that the cows would probably have had their second oestrus even before the start of the study. This was because first ovulation normally occurs around 25 days to 30 days postpartum (Schirar and Martinet, 1982).

vi CALVING TO CONCEPTION INTERVAL

The calving to conception interval for the years 1986, 1987, 1988, 1989, 1990, 1991 were 84±30, 79±24, 196±181, 108±60, 112±103, 79±34 respectively (Table 2, 4; Graph 4). The mean calving to conception interval was the average of the individual calving to conception intervals for a group of cows which was 79±34 days for 1986-1991.

TABLE 3 HERD FERTILITY 1992

i. Pregnancy rate to all service	41.07% (23/56)
Pregnancy rate to first service	47.22% (17/36)
ii. Oestrus detection rate	44.19% (19/43)
Oestrus cycle 1-17 days	5.88% (1/17)
Oestrus cycle 18-24 days	64.71% (11/17)
Oestrus cycle 25-35 days	17.65% (3/17)
Oestrus cycle 36-48 days	5.88% (1/17)
Oestrus cycle >48 days	5.88% (1/17)
iii. Services per conception	2.43 (56/23)
iv. Fertility factor	18.15
v. Percent Pregnant of served	63.88%
vi. Calving to first service	64.96 days
Standard deviation (Total of 23 cows)	19.51
vii. Anoestrus at 50d postpartum	5%
viii. Repeat breeders	6.25% (2/32)
ix Early embryonic death	15.63% (5/32)
x. Post-partum anoestrus >30 days	20.45% (9/44)
Post-partum anoestrus	57.3 days
Standard deviation (Total of 10 cows)	19.16
xi. Post-partum cyclicity	59.47days
Standard deviation (Total of 34 cows)	17.33

Assumptions:

i. Cows may have shown oestrus cycle earlier but were not observed for oestrus because investigation has not started.

ii .Some cows were not included in the calculations because they were without calving dates or not brought for the clinical examination.

iii. Some of the oestrous cycle have to be based on low progesterone level or clinical examination.

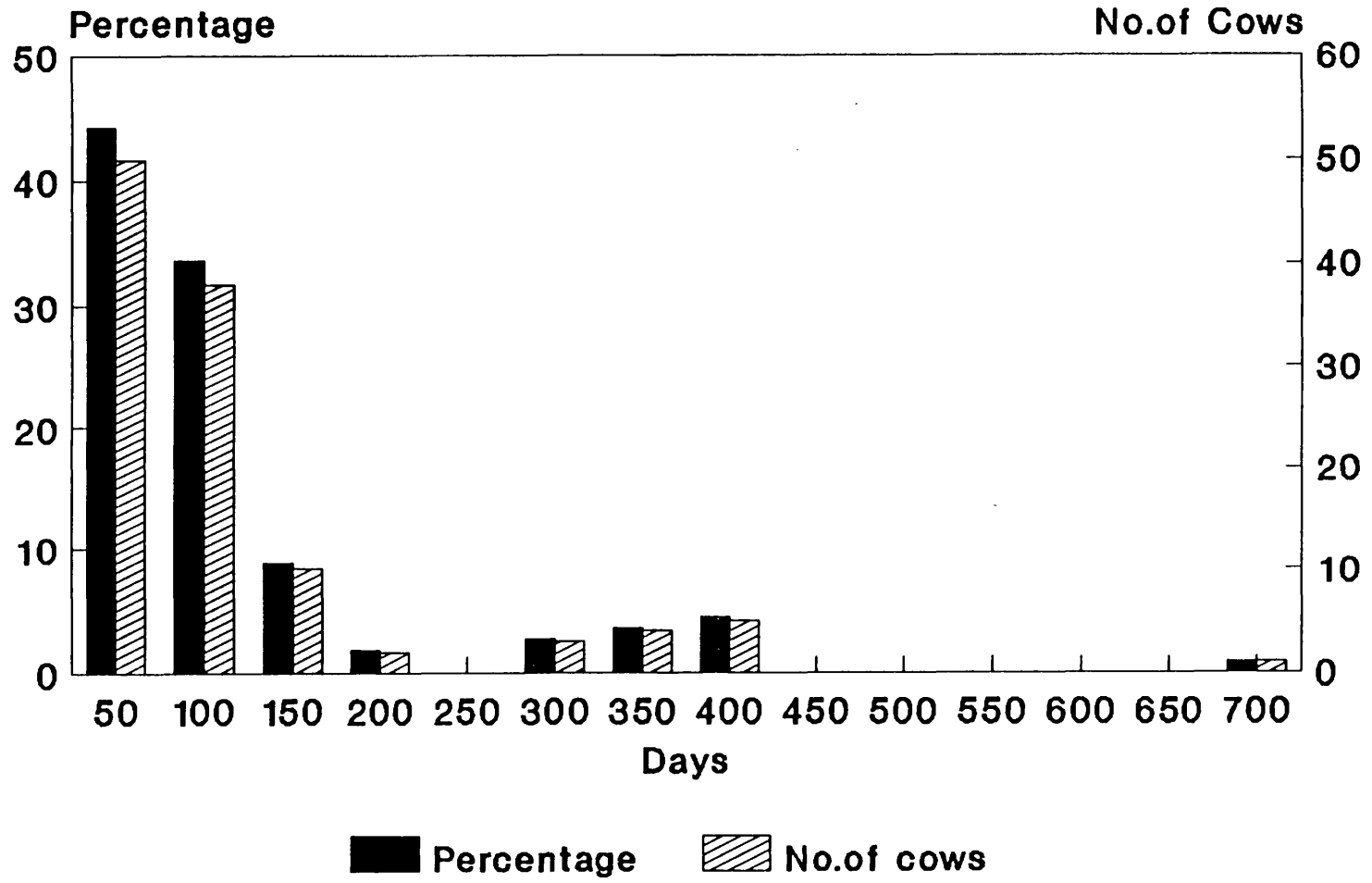
iv. The target date of service is assumed to be 60 days post-partum.

TABLE 4 CALVING TO CONCEPTION INTERVAL 1987-1992

YEAR	1987	1988	1989	1990	1991	1992
	116	98	299	41	45	74
	66	36	45	72	59	102
	80	70	48	74	79	200
	86	61	676	70	41	42
	83	81	378	129	62	75
	148	52	69	147	66	101
	131	95	306	101	76	106
	44	78	381	75	44	87
	49	72	52	135	117	38
	95	85	346	153	55	74
	60	93	76	175	358	59
	60	130	380	101	72	65
	73	-	399	45	41	59
	90	-	140	94	87	77
	-	-	39	119	70	71
	-	-	41	90	69	81
	-	-	38	83	167	55
	-	-	62	63	402	51
	-	-	84	328	82	96
	-	-	43	65	296	71
	-	-	347	144	77	-
	-	-	58	88	109	-
	-	-	-	93	-	-
AV.	84.36 ±30.29	79.25 ±24.28	195.77 ±180.81	108.04 ±59.62	112.45 ±102.76	79.20 ±34.29
NO.COW	14	12	22	23	22	20

MEAN CALVING TO CONCEPTION INTERVAL 79.20±34.29

GRAPH 4 AVERAGE CALVING TO CONCEPTION
1987-1992



Total of 113 cows in Pollok farm

vii CONCEPTION TO 1ST SERVICE AND PREGNANCY RATE

In table 3, the pregnancy rate to 1st service was found to be 47% for the current year (Graph 5). The pregnancy rate to all service was 41%. The percentage pregnant of cows served was found to be 64% and services per conception was calculated to be 2.43 for the current year. The fertility factor was 18.15.

viii CU-SUM OF PREGNANCY

Graph 6 showed the cumulative sum of pregnancy for the period of study. It was noted that the conception rate was poorer in the months of May and June which might be correlated with the changes in feeding, environment, management or mating procedures.

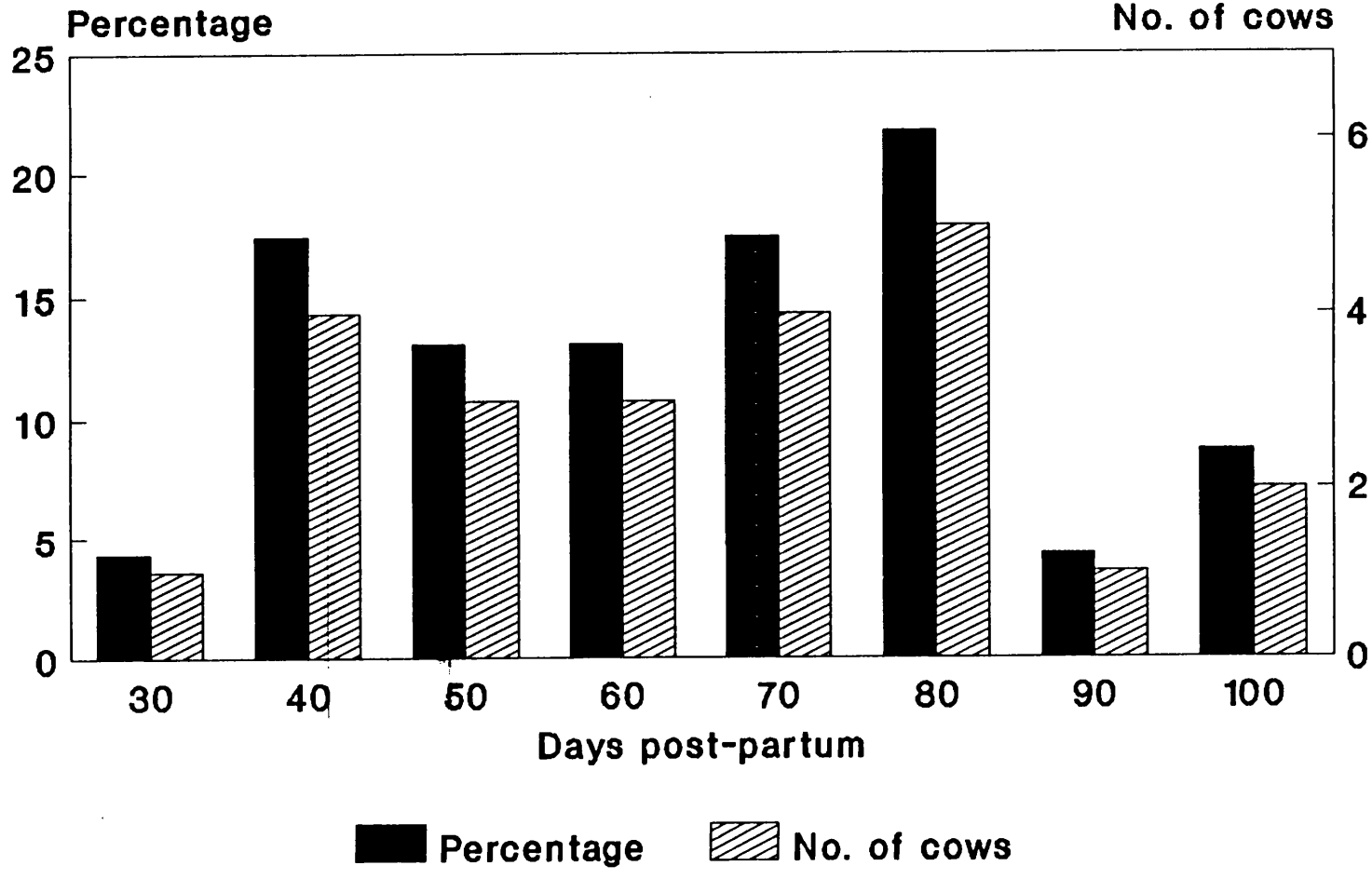
ix ULTRA-SONIC SCANNING

An illustration of ultrasonography indicating pregnancy at 3 months and a normal corpus luteum were shown in plates 3 and 4 respectively.

x PROGESTERONE ASSAY

The current studies showed there was a 80.8 % of correlation between the presence of CL to progesterone concentration and 19.2% of palpations were inaccurate (Table 5). A proportion of cows considered to have a CL present (7.4%) on rectal palpation showed relatively low levels of plasma progesterone. Likewise, the reverse also happened, in that 11.8% of the high progesterone concentration disagreed with the rectal palpation findings. It was found that 5% of the cows had not cycled even 50 days after calving (Graph 7, 8).

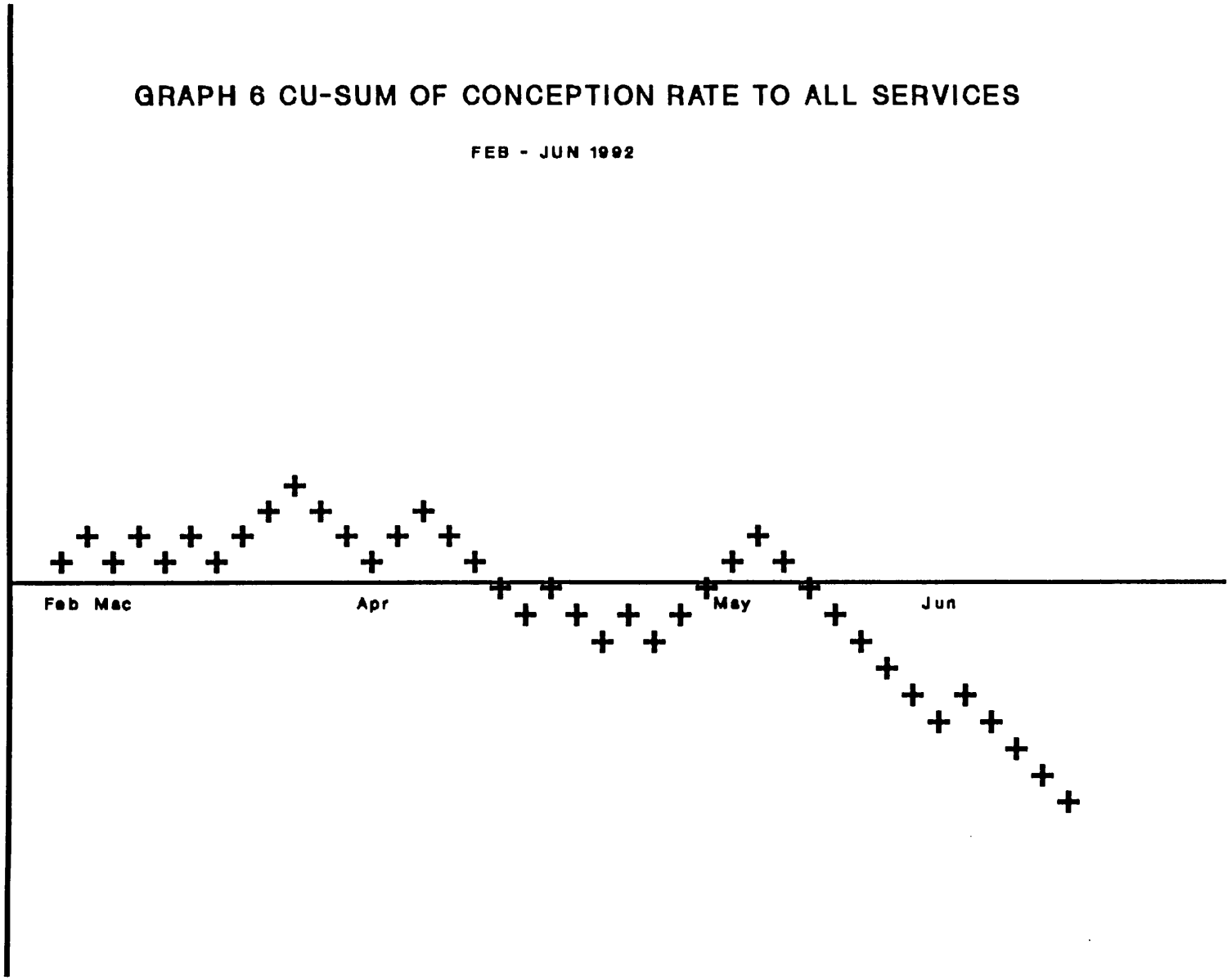
GRAPH 5 CALVING TO CALCULATED FIRST SERVICE 1992



Total of 23 cows in Pollok farm

GRAPH 6 CU-SUM OF CONCEPTION RATE TO ALL SERVICES

FEB - JUN 1992



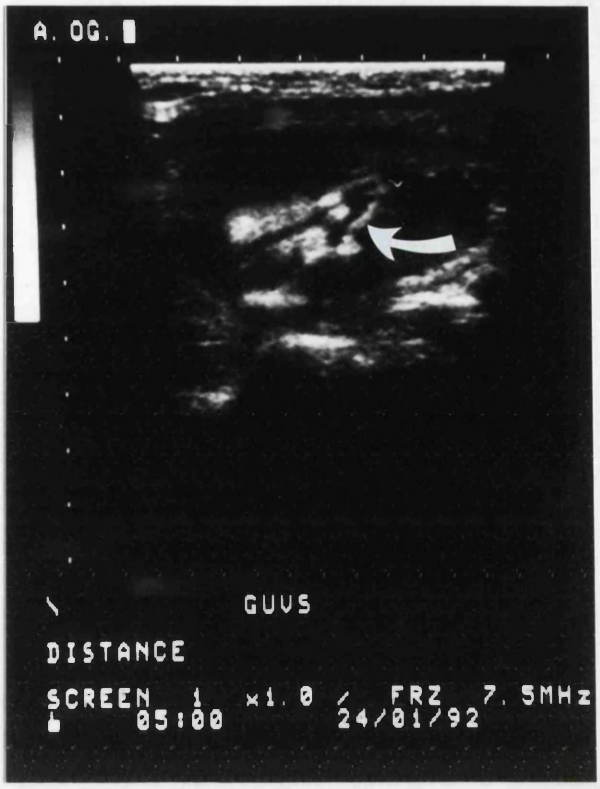


PLATE 3 ULTRASONIC SCANNING OF PREGNANCY AT 3 MONTHS. SEX FEMALE FETUS. f= foetus

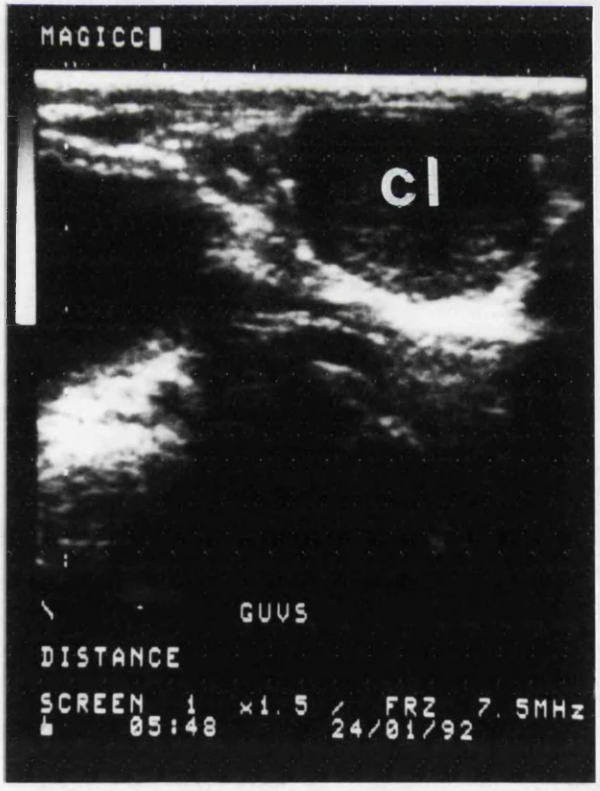


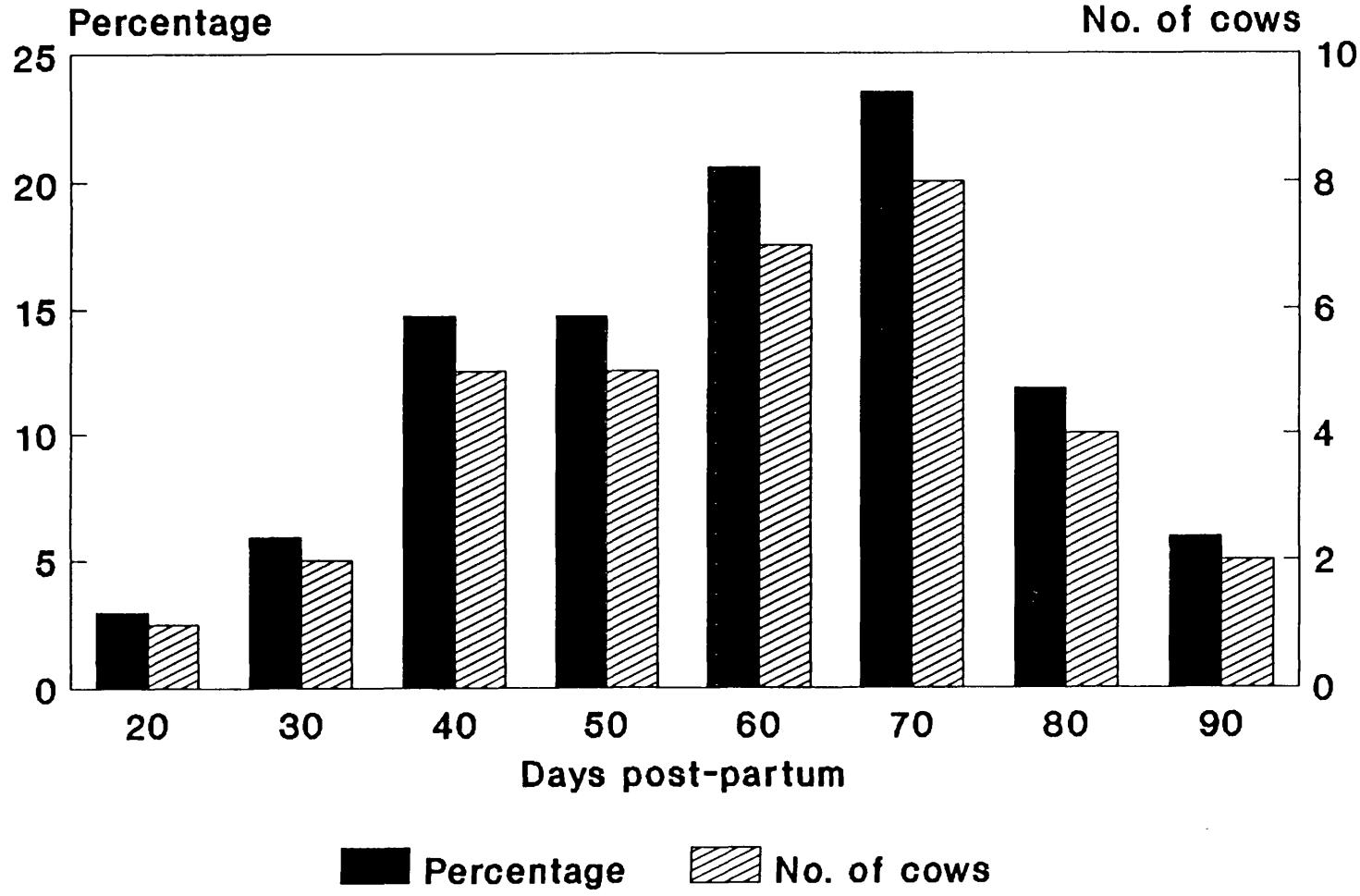
PLATE 4 ULTRASONIC SCANNING OF OVARY. NORMAL CL.

TABLE 5 RELATIONSHIP OF CL AND PROGESTERONE LEVELS

MANUAL	PROGESTERONE	
CL	FUNCTIONAL	NON FUNCTIONAL
82	80.8% (80/99)	7.4% (6/82)
NO CL	FUNCTIONAL	NON FUNCTIONAL
17	11.8% (2/17)	88.2% (15/17)

GRAPH 7 POST-PARTUM CYCLICITY IN BHC

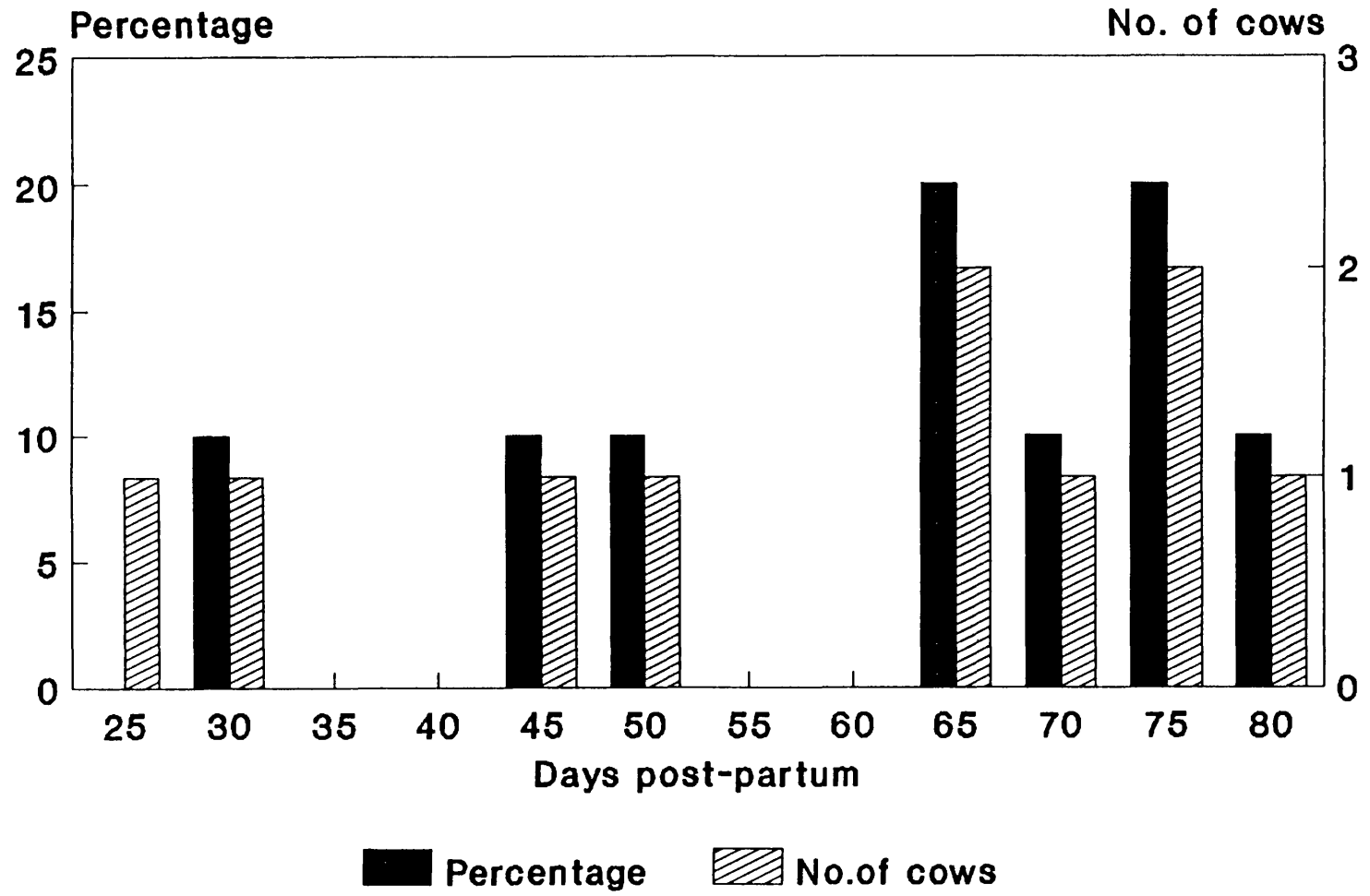
1992



Total of 34 cows in Pollok farm

GRAPH 8 POST-PARTUM ANOESTRUS IN BHC

1992



Total of ten cows in Pollok farm

4.2 FARM 2

i ABNORMALITES OF REPRODUCTIVE TRACTS. ABATTOIR SPECIMENS.

It was found that 67% of the reproductive tracts were abnormal (table 6). The gross morphological abnormalities were cervical strictures (16.7%), oviductal blockage (17%), small periovarian cyst (8%), ovarian adhesions (33%) and double os cervix (8%), (table 6; plates 5 & 6).

ii UTERINE CULTURE

The uterine cultures showed that 92% of the reproductive tracts from the abattoir specimens harboured no bacteria. Only one sample contain 6 colonies of streptococci isolated in microaerophilic conditions. All of the samples were free from Salmonella and Haemophilus somnus.

iii OVIDUCT PATENCY TEST

Ten (83%) of the reproductive tracts showed no oviductal blockage. However, there was one case each which showed bilateral and unilateral oviductal non-patency (Table 6).

TABLE 6 ABNORMALITIES OF REPRODUCTIVE TRACTS OF ABATTOIR SPECIMENS FROM FARM 2.

MORPHOLOGICAL ABNORMALITIES	%
Cervical stricture	* 16.67 (2/12)
Double os cervix	8.33 (1/12)
Bilateral oviductal blockage	8.33 (1/12)
Unilateral oviductal blockage	8.33 (1/12)
Mild ovarian bursa adhesion	33.33 (4/12)
Small periovarian cyst	8.33 (1/12)
Tracts with abnormalities	58.33 (7/12)

* There were no noticeable mucometra of the uterus with cervical stricture.

TABLE 7 INCIDENCE OF 1/29 CHROMOSOMAL TRANSLOCATION

TYPE OF TRANSLOCATION	NO. OF ANIMALS		TOTAL
	FARM 1	FARM 2	
HETEROZYGOUS 1/29	5 (11.4%)	3 (12%)	8 (11.7%)
HOMOZYGOUS 1/29	0	0	0
NO. ANIMALS ANALYSED	44	25	69

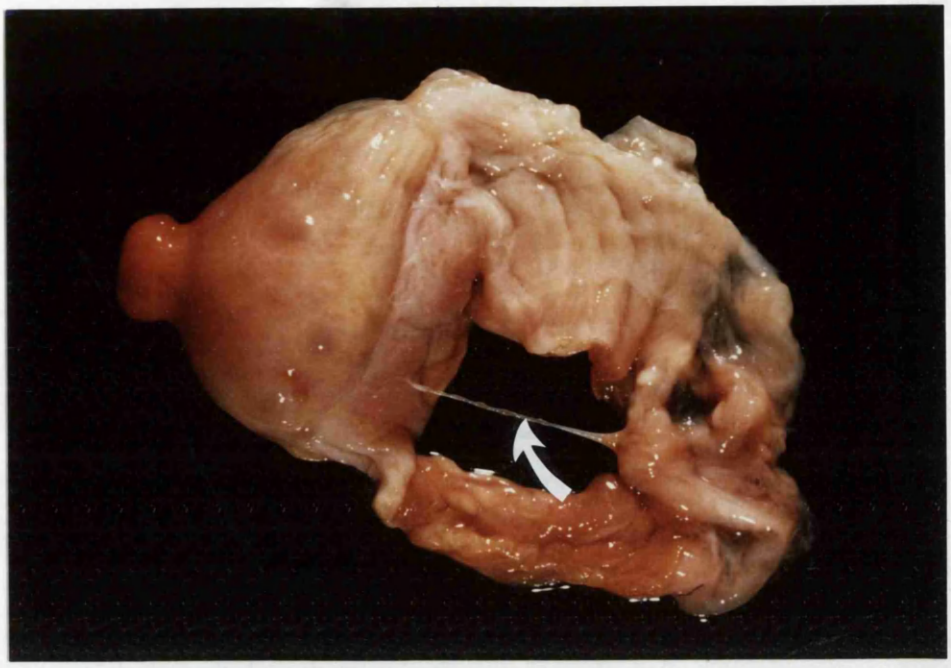


PLATE 5 FIBRIN TAGS IN OVARIAN BURSA

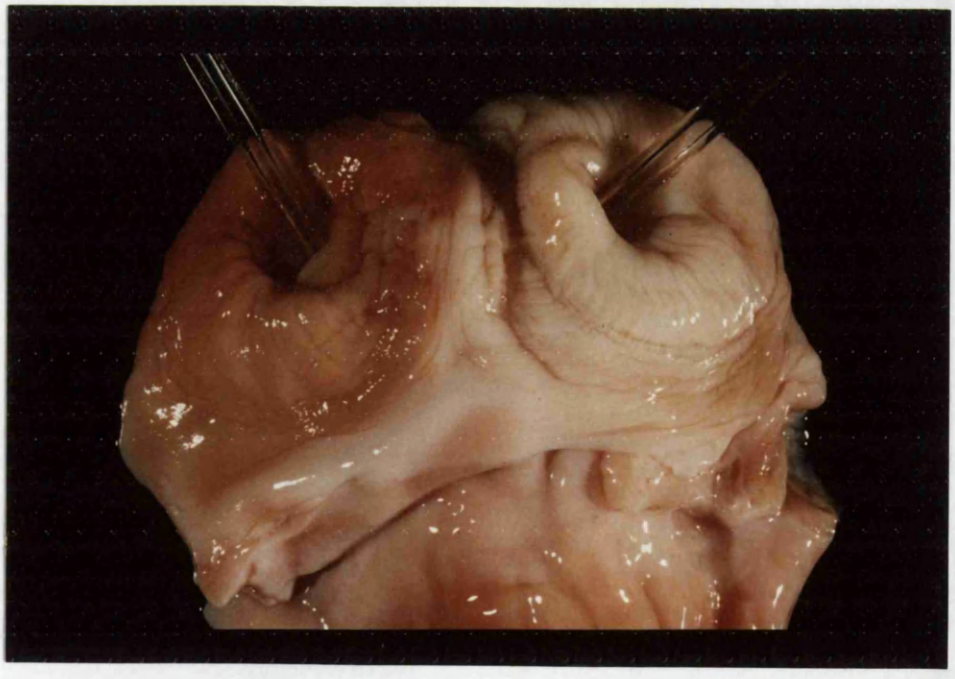


PLATE 6 DOUBLE OS CERVIX

iv UTERINE HISTOLOGY

The luminal epithelium was frequently lost from the tissue specimens because of the method of sectioning and processing of the tissue. The histology sections showed normal endometrium with no obvious inflammation (plates 7) and normal oviductal structures (plate 8).

4.3 CHROMOSOME ANALYSIS

The analysis showed 11.36% and 12% of the cows in farm 1 and farm 2 respectively were heterozygous carriers of Robertsonian translocation (Table 7; Plates 9-14), a total of eight animals, comprising seven cows and one bull out of 69 cows analyzed. The relationship postulated is shown in figure 1. The pedigrees of the affected translocation animals are shown in figures 2 to 9.

The animals heterozygous for Robertsonian translocation 59,XX,t were denoted by the symbol 't+' and those animals free from the translocation were denoted by 't-'. There were no animals diagnosed homozygous for the translocation.

The Robertsonian translocation involved chromosomes numbers 1 and 29 which were identified by C and G banding techniques (plate 13 ,14).

The C-banding caused the constituitive heterochromatin to stain darkly and it showed the translocation to be monocentric as shown in plate 13.

In G-banding, the banded regions of the chromosome contained proteins which are resistant to proteolytic enzymes (Ridler and O'Hara, 1972). The non-histones interacted with the dye and contributed to the banding

pattern (Sumner, 1972). The G positive bands are rich in protein disulphides whereas sulphydryls predominate in G negative bands (Sumner, 1974). These banding patterns corresponded with the identification and position of the chromosome number 1 and 29 in the karyotype as shown in plate 14, although the smaller chromosome was difficult to identify positively. Thus, it could be tentatively confirmed to be chromosomes 1 and 29 based on the characteristics of the bands. This karyotyping followed the standard system of nomenclature adopted at the International Conference on the Standardisation of Banded karyotypes of Domestic Animals, 1980 (Ford et al, 1980).

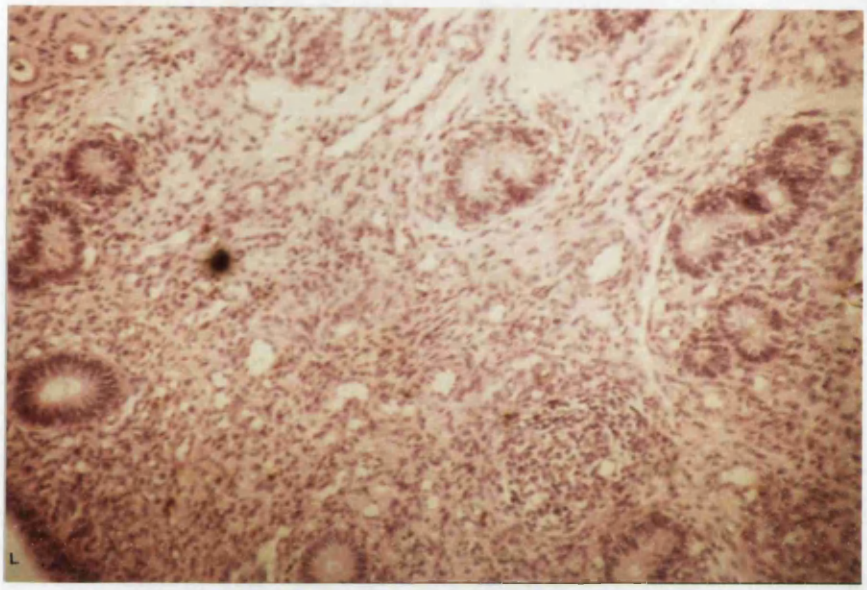


PLATE 7 HISTOLOGY SECTION OF UTERUS.
NORMAL ENDOMETRIUM (X 100) l = lumen

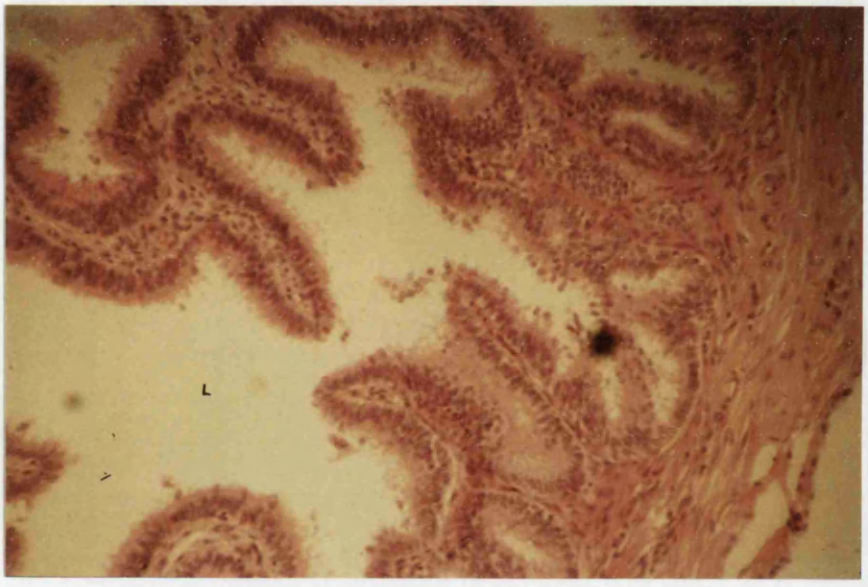


PLATE 8 HISTOLOGY SECTION OF OVIDUCT
NORMAL OVIDUCT (X 100) l = lumen

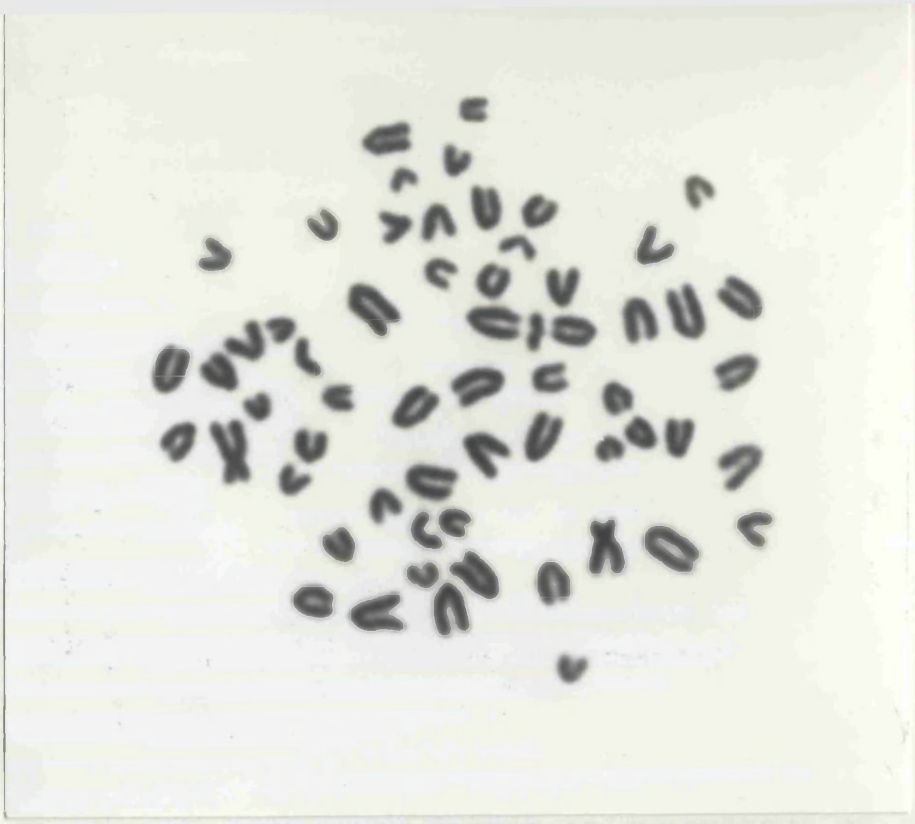


PLATE 9A METAPHASE SPREAD OF NORMAL CHROMOSOME OF A COW

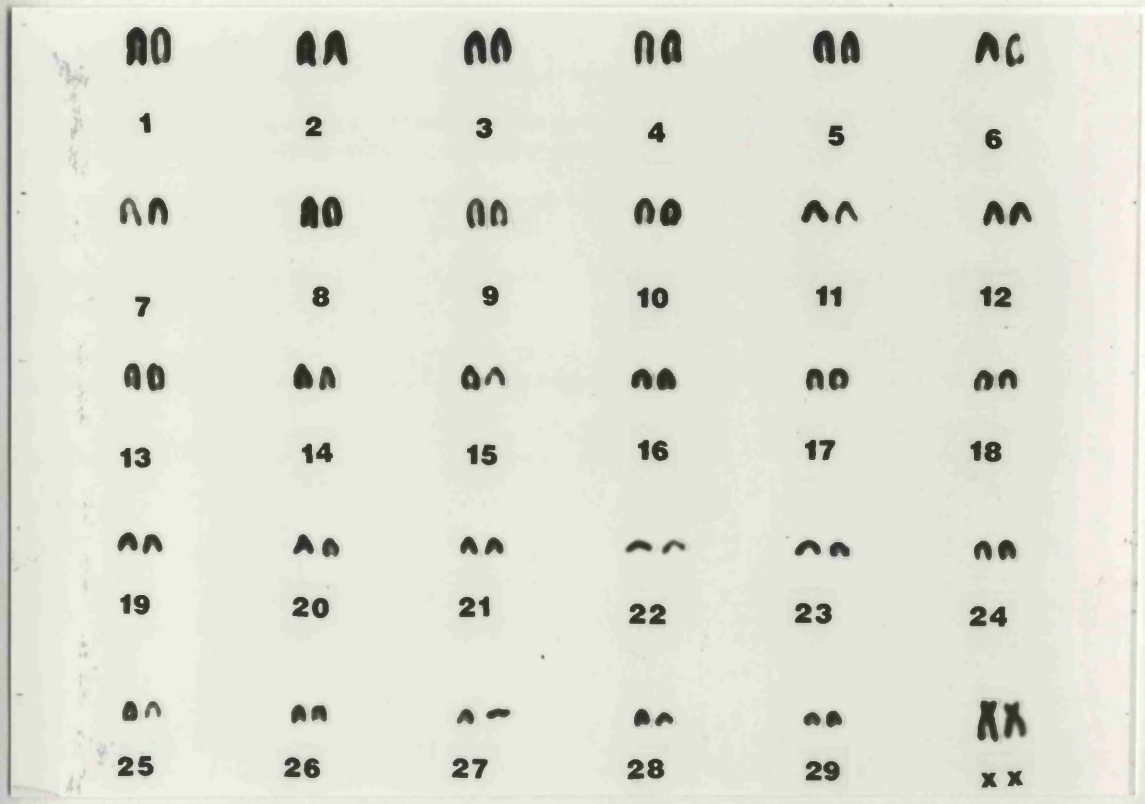


PLATE 9B NORMAL CHROMOSOMAL KARYOTYPE OF A COW (X 1000)

PLATE 10A METAPHASE SPREAD OF CHROMOSOME OF THE COW HETEROZYGOUS FOR 1/29 TRANSLOCATION

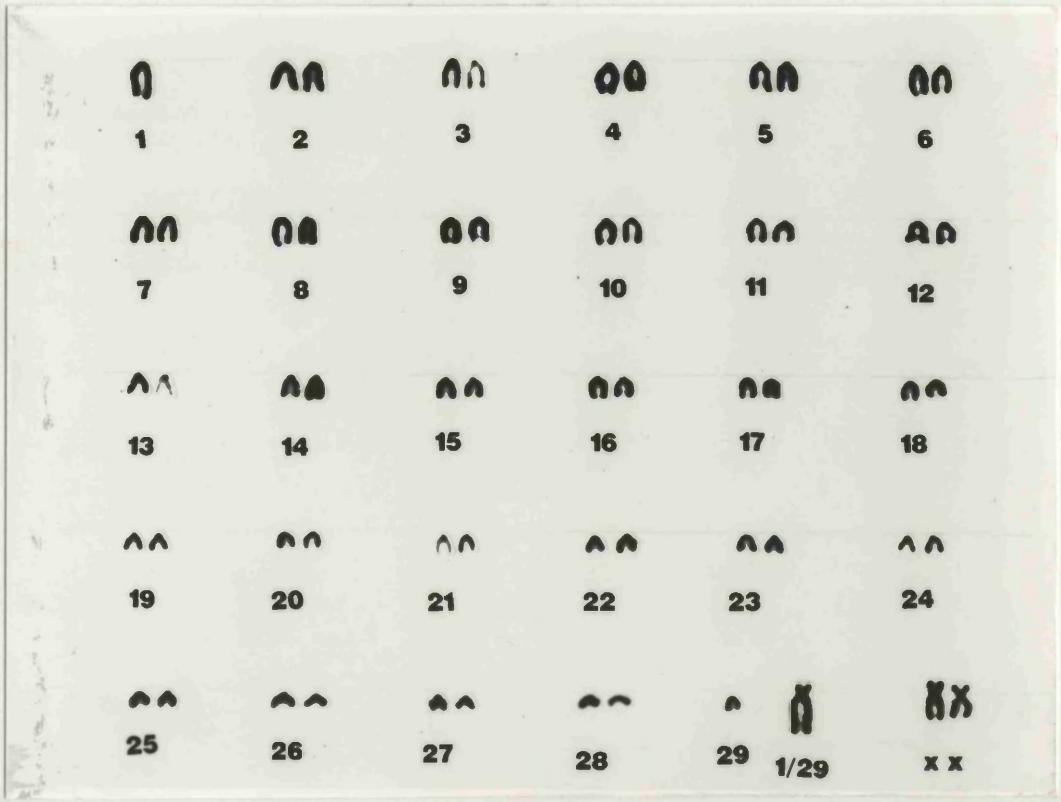


PLATE 10B CHROMOSOMAL KARYOTYPE OF THE COW HETEROZYGOUS FOR 1/29 TRANSLOCATION (X 1000)

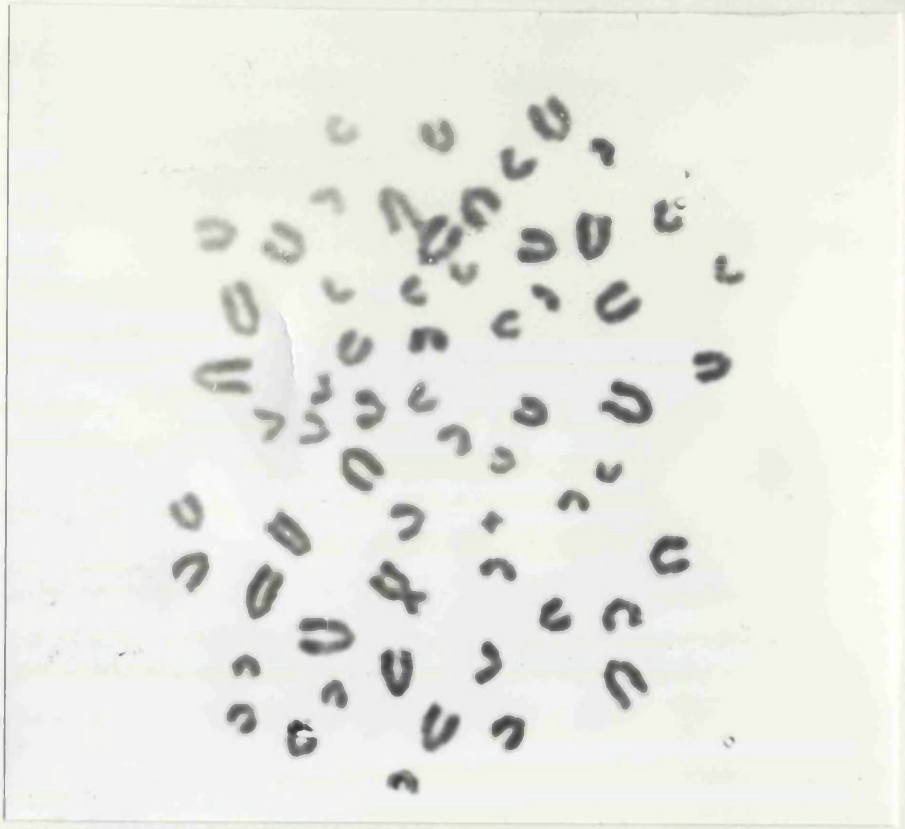


PLATE 11A METAPHASE SPREAD OF CHROMOSOME OF A NORMAL BULL

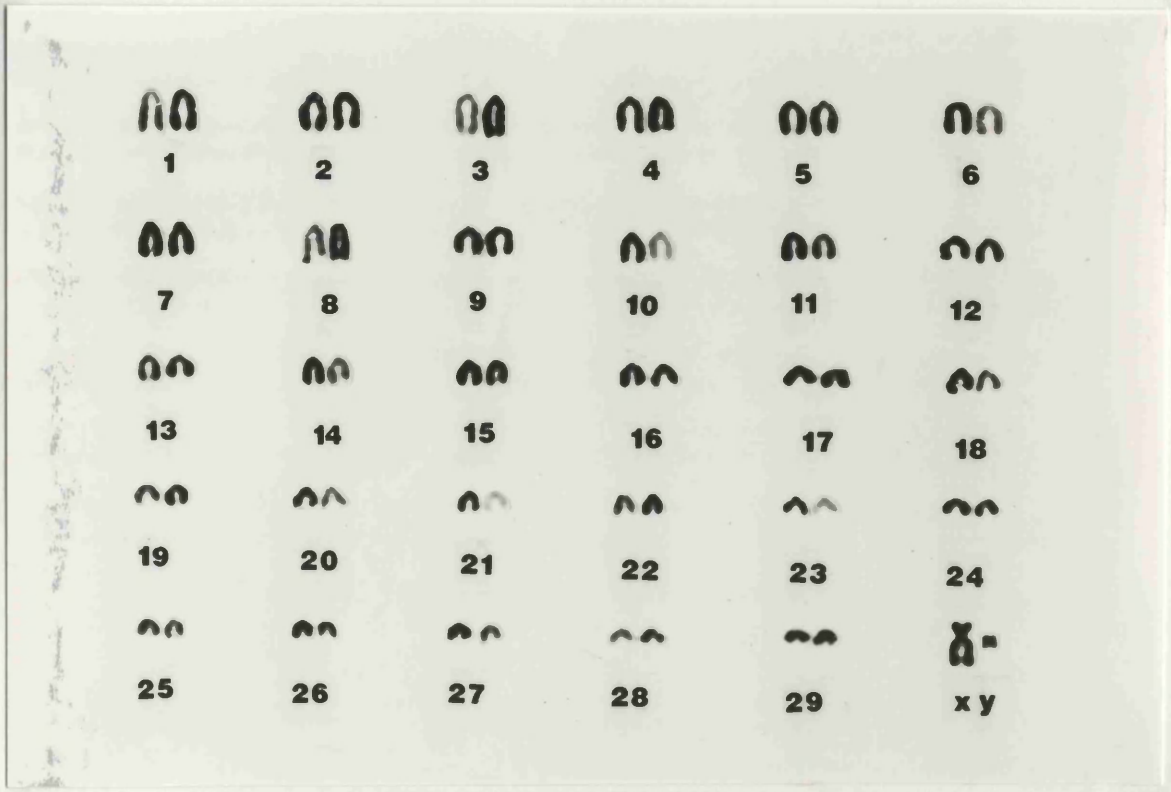


PLATE 11B NORMAL CHROMOSOMAL KARYOTYPE OF A BULL (X 10000)

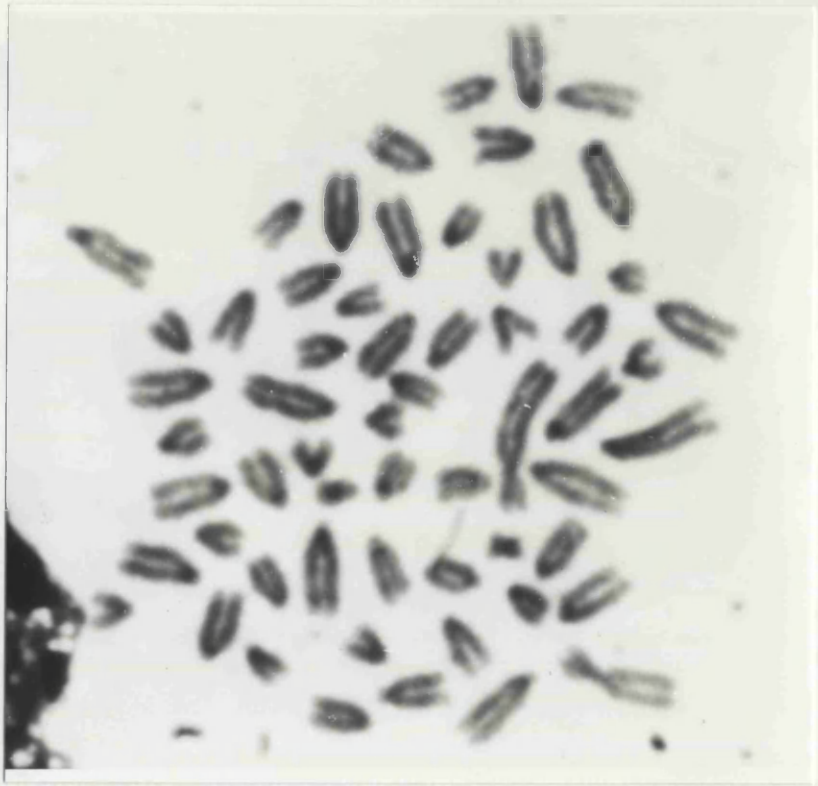


PLATE 12A METAPHASE SPREAD OF A BULL HETEROZYGOUS FOR 1/29 TRANSLOCATION

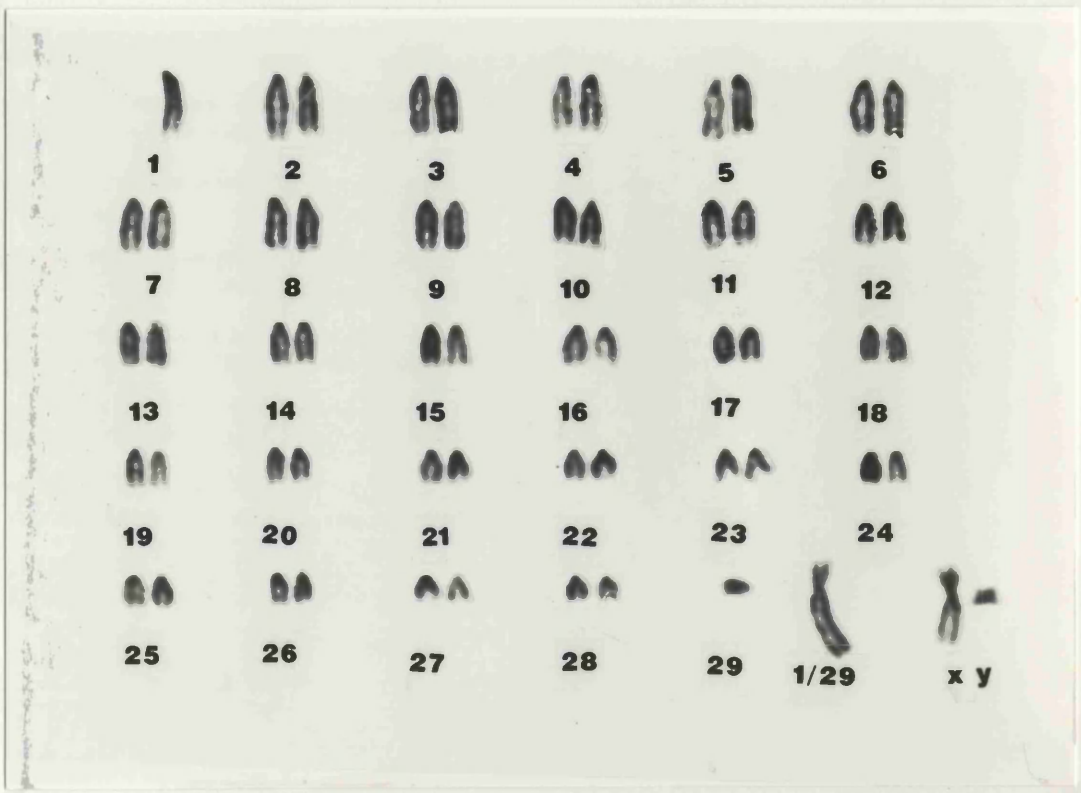


PLATE 12B CHROMOSOMAL KARYOTYPE OF A BULL HETEROZYGOUS FOR 1/29 TRANSLOCATION (X 1000)

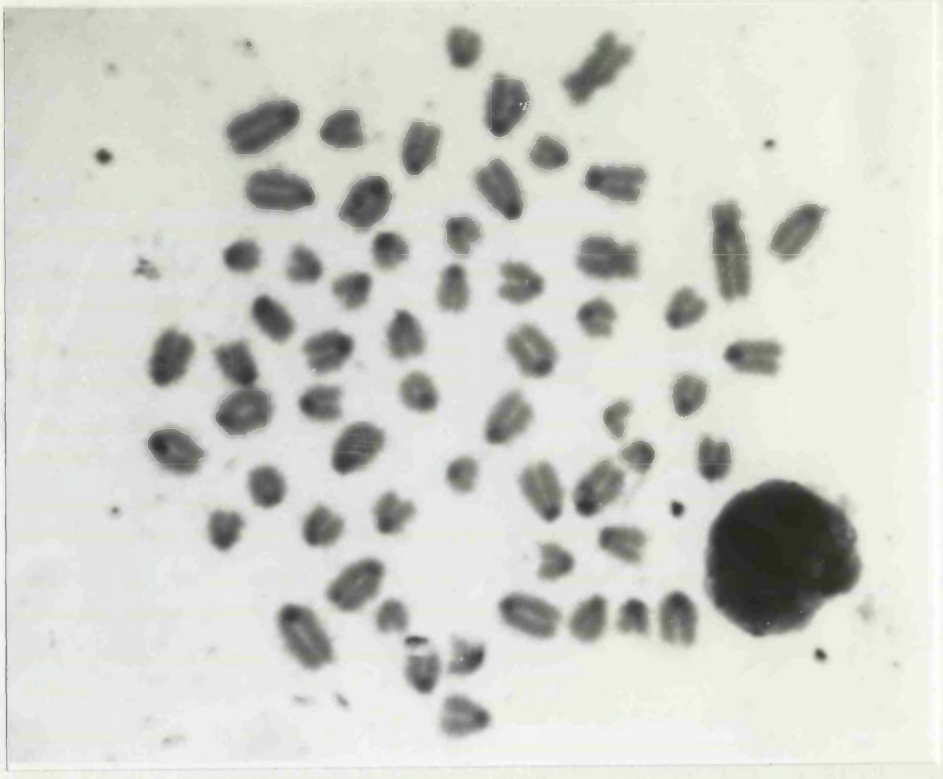


PLATE 13A METAPHASE SPREAD OF THE CHROMOSOME WITH C BANDING OF THE COW HETEROZYGOUS FOR 1/29 TRANSLOCATION

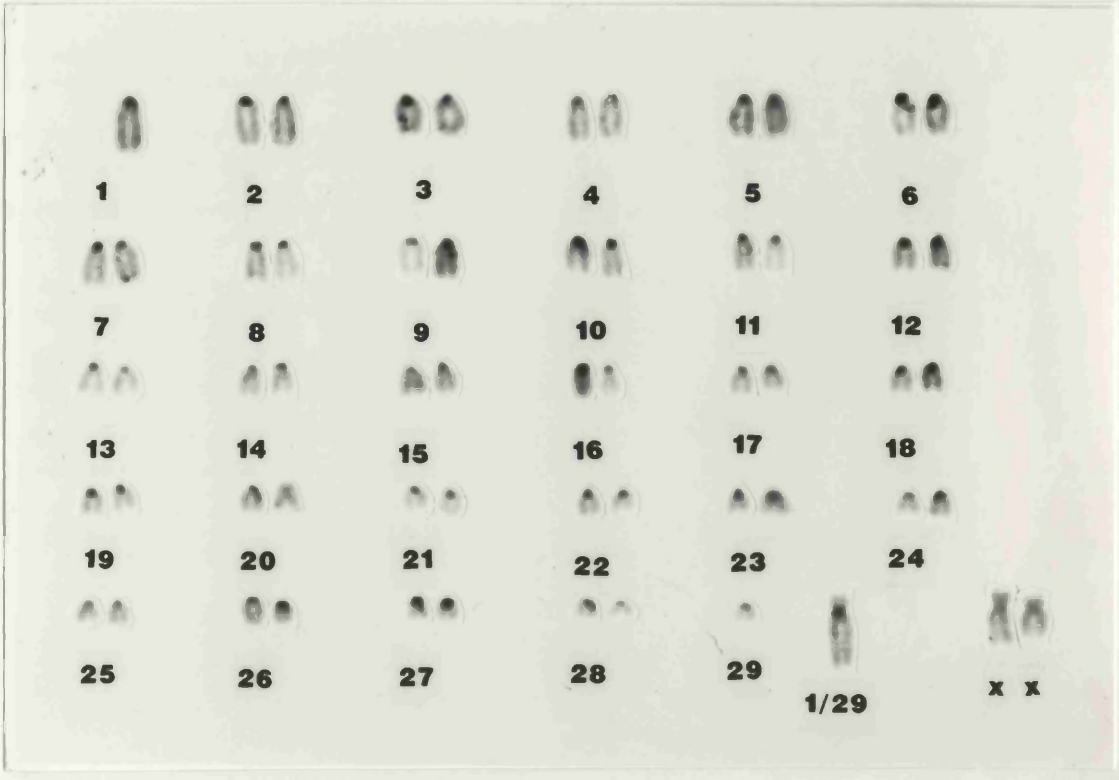


PLATE 13B C BANDING OF THE COW HETEROZYGOUS FOR 1/29 CHROMOSOMAL TRANSLOCATION (X 1000)

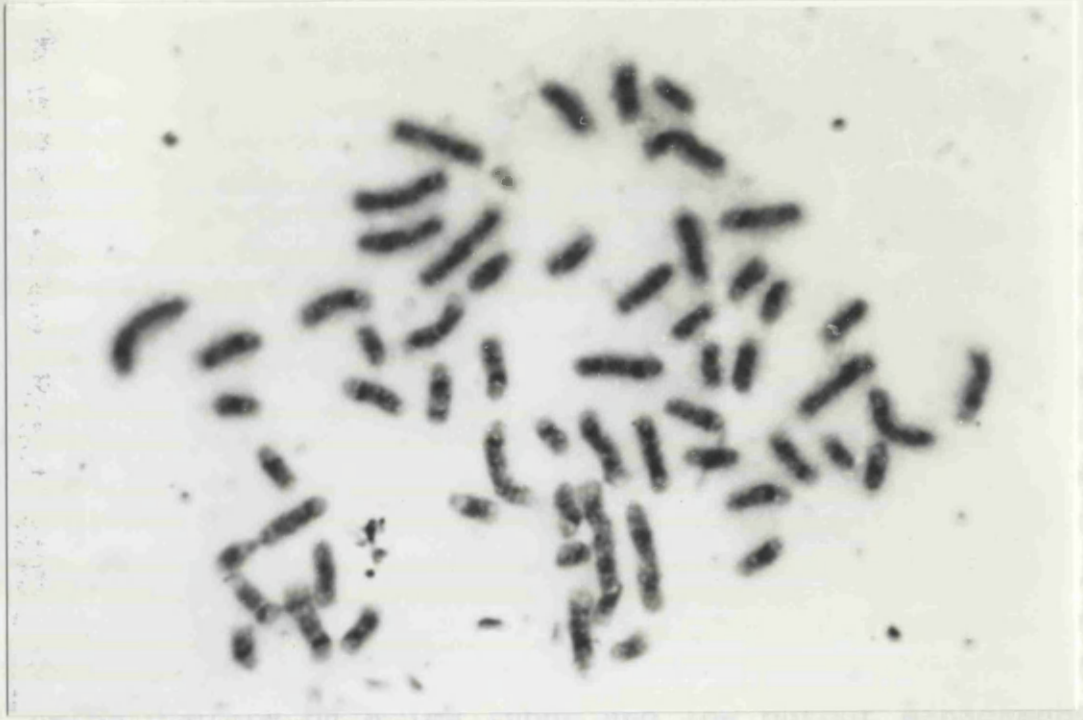


PLATE 14A METAPHASE SPREAD OF THE CHROMOSOME WITH G BANDING OF THE BULL HETEROZYGOUS FOR 1/29 TRANSLOCATION

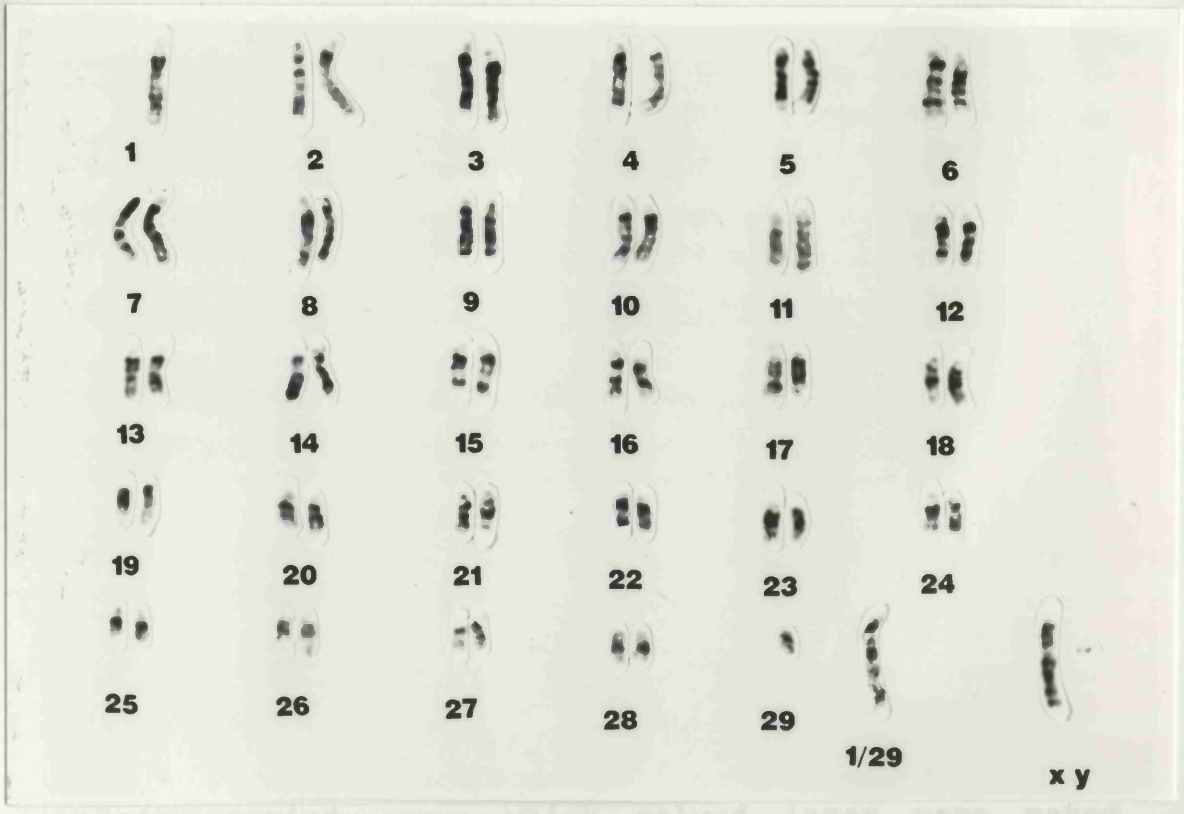


PLATE 14B G BANDING OF THE BULL HETEROZYGOUS FOR 1/29 TRANSLOCATION (X 1000)

CHAPTER FIVE

5.0 DISCUSSION

Normally, beef cattle are not as intensively managed as dairy herds. There is economic importance attached to elaborate recording systems which are essential in the dairy herd for proper control of reproductive and milk production. Thus, many of these parameters used in the dairy farm such as submission rate, oestrous detection, interval to first oestrus, interval to first breeding, days open, first service conception rate, services per conception, are often not used to measure reproductive efficiency in the beef herd. Many beef herds operate on a low input and low output management system where the criteria used to measure reproduction and production is the number of calves weaned or sold. This is because it is a measure of breeding efficiency, calf survival at birth and nursing survivability. Other measures of breeding efficiency applicable are percent number of cows cycling during first 21 days breeding, first service conception rate, percent number of cows pregnant at 60 days of the breeding season, calf mortality and weaning weights. However, for the purpose of this study the dairy cattle parameters were used wherever possible even though it might not have been very accurate, but would provide a reasonable assessment of the fertility.

5.1 FARM 1

i CALVING PATTERN

Since most calvings occurred in spring, the cows were often mated early after calving as the mating season was of limited length. In order to achieve a closer calving period, cows which calved later were mated sooner after calving than the early calvers. Sometimes, cows that calved down later were barren until next

season because of a management decision not to mate them in order to fit in better with the next year's mating season.

There was a noticeably widespread calving period during the current year (1992). This will be even wider in 1993 because of late calvings and repeat breeding. Shortening the calving season in Spring is desirable for many reasons, for example the age and weight of calves would be higher at sale and calf management would be much easier in groups. However, any treatment or culling to shorten the calving season would add considerably to the cost of production. Ideally, the cow should calve shortly after the commencement of the calving season. Normally, calves born late in calving season do not have a high average daily gain compared with calves born early in the calving season. Thus, it was the wish of the breeder to have his calvings earlier, namely at the end of winter, a month or so before the normal calving season in Spring. In this way, the calves would be older, heavier and therefore more saleable during the Autumn sales. These calves will grow faster because the dams produced more milk for the suckling calves as they gained access to better pastures in the spring. Furthermore, the registration of the breed was in September which will provide time for these calves to gain their maximum potential weight before the sale. Above all, the calves will be bigger than their contemporaries for competition in the show business. By contrast, Autumn born calves would be too young to be sold during the first autumn season and too small to compete with their contemporaries at cattle shows. These calves grow poorly because their dam will have poorer milk production because of poorer pastures. Selling them earlier would means a loss in potential body weight gain of the calves. A trial to shift the season of

calving to the end of winter was undertaken. However, this shift was slow because of environmental influence on the nutrition of the dam when ample forage was available during Spring. It has been reported that Spring calvers undergo longer intervals from calving to first ovulation than do autumn calvers (Bulman and Lamming, 1978). This was because photoperiod played some role in seasonality of reproductive activity. Similarly, season of birth would affect age at first oestrus in heifers. It was found that calves born in spring reached puberty two months earlier than those born in autumn (Peters and Ball, 1987).

ii CALVING INTERVAL AND CALVING INDEX

The calving interval and index were good measures for assessing fertility in purely retrospective studies. The results showed that there was a wide range of calving intervals from 250 to 650 days and a higher calving interval in 1989 with significant difference of $p=0.002$. This was because of the change in management of the animals with the change in staffing and also not all cows were included in this calculation as some were sold after calving. Short calving intervals could have occurred because of a rapid rate of involution of the uterus due to the suckling effect. However, it was difficult to reconcile this relationship with the ovarian activity. This was because onset of ovulation and oestrus in dam are supposedly delayed by suckling (Peter and Riley, 1982). The cows in farm 1 in the past have had calving to conception intervals of 30 days. The reasons suggested could be that the dam had poor milk production or that the calf had died, although this may not the case on this farm.

Long calving intervals could have resulted from policy decisions when repeat breeder cows were left barren

until the next breeding season. Longer calving intervals predispose to a wider spread of calving thus prolonging the calving season. There was an improvement in the calving interval over the years as evidenced by the trend. This could be due to improved management and awareness. Shorter calving intervals could be expected from cows that were served soon after calving but the pregnancy rate will be lower. This could have had a deleterious effect on the cow and the calving pattern. The average calving interval became the calving index.

iii OESTROUS DETECTION RATE

The oestrous detection rate of 44% was considered satisfactory in this study because the herdsman had other duties to perform. It was generally accepted that the detection rates are rarely better than 60% and in many cases are less than 50% (Arthur et al, 1989). In dairy herds, because of closer observation, the result would be expected to be better, namely 82-97% for trained staff and 67% for untrained staff (Esselmont, 1974). Normally, at first postpartum ovulation only 50% of the animals showed signs of oestrus while at subsequent ovulations, 94% are detected (Arthur et al, 1989). It was also noted that oestrus detection was higher in those cows that calved earlier than those that calved later. This was probably because in the former, the presence of more non-pregnant cows might ensure greater interaction during oestrus which improved its detection. Cows which calved early would have had a longer time interval to be served compared with those that calved late in the season.

In order to aid in oestrous detection, the bull should be fitted with a marking device. The bull to cow ratio was less than 1:20 which was satisfactory (Table 8).

TABLE 8 MATING BULLS, ABORTION, CULLING RATE AND CALF MORTALITY

Year	1986	1987	1988	1989	1990	1991	1992
Mating bulls	1	1	1	2	2	4	4
No. breeders	45	45	45	45	45	55	55
Ratio	1:45	1:45	1:45	1:23	1:23	1:14	1:14
No. calvings	19	23	14	28	26	39	41
Abortion	1	2	0	1	5	2	5
% Abortion	5.26	8.70	0.00	3.57	19.23	5.13	12.20
Calf Mortality	0	1	1	1	1	2	0
%	0	4.35	7.14	3.57	3.85	5.13	0
No. Culled	16	6	5	10	5	9	5
% culled	35.55	13.33	11.11	22.22	11.11	16.36	9.09

It was important that the bull to cow ratio be low otherwise the bull might be overworked resulting in loss of libido. This would result in some of the cows that were on heat not being mated which would obviously lower the oestrus detection rate.

The interoestrus intervals were found to be 6%, 65%, 18%, 6%, 6% for 1-17 days, 18-24 days, 25-35 days, 36-48 days and more than 48 days respectively. These values were obtained from only 11 cows which was a small number from which to draw any conclusion. This small sample size was because of difficulty in oestrous detection and in determining their cyclicity. In a well managed herd, the values obtained were 12%, 53%, 15%, 10% and 10% respectively (Anon, 1984). In comparison, this indicated the herd was managed reasonably well with quite a good level of accurate detection of oestrus as the majority of the cows were detected on oestrus within 18-24 day period. Since the majority of cows return to heat within the normal oestrous period, it could be suggested that there was no apparent functional hormonal disorder in the cows. However, if early embryonic death occurred before day 13, the cow would also show heat within the normal oestrous cycle (Ayalon, 1981). The interoestrus interval of more than 24 days could be associated with late embryonic death. Cycle lengths equal to two normal oestrus cycles were open to a variety of interpretations. It was clear therefore, that exact interpretation of oestrous intervals had to be evaluated together with other parameters. It was difficult to determine at this stage if the cow has failed to conceive or if conception followed by embryonic loss.

The same bulls were used in the herd for the past five or six years except for two bulls which were purchased last year. Presumably, this might lead to some

inbreeding unless the matings were well regulated in preventing sire-daughter matings. This would lead to an increase in the services per conception as was reported in one Holstein herd (Woodward and Graves, 1946). This might be one of the factors that could contribute to the cause of infertility.

iv CLINICAL FINDINGS

There was a good relationship with 81% of plasma progesterone concentrations agreeing with the identification of a palpable CL (Table 5). This result compared well with other researchers who recorded 77% in 142 examinations (Boyd and Munro, 1979). However, it has been reported that a CL can be physically present in the absence of high progesterone level (Pope et al, 1969). Inaccuracies occur in the detection of the presence of CL when large follicles or cystic structures are present (Dawson, 1975) or when the cow is at either end of the oestrous cycle.

v PREGNANCY RATE

The pregnancy rate of 41% was lower than the normal value of 55% (Arthur et al, 1989). This could be attributed to the lower oestrus detection rate, which was probably due to the fact that oestrus detection was carried out only on an ad hoc basis for the purpose of this study as natural mating by the bulls was practiced. The submission and pregnancy rates were quite reliable measures for the assessment of fertility levels but these might vary during the breeding season. As expected, oestrous detection and pregnancy rates were likely to be higher in the early compared with the later part of season. This was because the nutrition was likely to be better and exhibition of

oestrous signs was more obvious due to the smaller numbers of cows which were pregnant.

vi FERTILITY FACTOR AND PREGNANCY RATE

It might not be feasible to aim for a 365 day calving interval with a fertility factor of less than 35 because the percentage of barren cows was high. The pregnancy rate to 1st service was 47% and overall pregnancy rate was 41% which was lower because the latter included cows which had received many services. These values were slightly lower than dairy herds which had a mean values of 60% and 58% respectively (Arthur et al, 1989). The number of services per pregnancy was 2.43 which indicated a high number of services for a conception to take place. The percentage pregnant of those served was 64% which did not necessarily mean that the conception rate was good since they might had been served several times.

To improve the pregnancy rate, the bulls should be regularly checked and monitored for fertility. Bull performance was just as important and partial or total infertility will seriously damage the reproductive performance of the herd. If bull was sub-fertile it would take a long time before it was being detected, resulting in loss of time and lowered conception rate. Similary, cows with reproductive problems would also remain undetected for a long period unless pregnancy diagnosis was carried out early. It could be of considerable value when introducing new bulls to test them for disease and semen quality. In the present study, bull investigations were suggested on numerous occasions but there appeared to be little enthusiasm to allow semen assessment to be conducted on these bulls.

vii CALVING, WEANING RATE AND CALF WEIGHTS

As the calf crop is virtually the sole product of a beef herd, the number of calves born is the major reflection of the reproductive performance. The calving rate would be slightly lower than the pregnancy rate of 47% for the current year and the number of calves born per year had increased throughout the 6 years (Table 1; Graph 1). There was no record of postnatal calf deaths up to weaning which reflected a good calf management standard which was commendable. Thus, the weaning rate was equivalent to the calving rate.

Since most of the calvings were in Spring, the weight gain and growth rate of the calves were better because the dams calved in time to utilise the spring pastures. This in turn enabled the dams to produce enough milk to feed the calves to grow faster and this was reflected in better calf weight. Thus, the weights of calves in relation to their ages were higher at sale.

viii CULLING RATE

The overall culling rate was 17% (Table 2, 8) because of sale of breeders for economic reasons. There was hardly any culling based solely on infertility problems. In dairy herds, the annual wastage from sterility was found to be about 5% of the population (Esselmont et al, 1985). The difference in culling rate could be for various reasons and there is also a difficulty in classifying the cause of disposal. Nevertheless, the culling rate here was comparatively low as high culling rates are rarely cost effective because replacement cost are much greater. High culling rate of repeat breeders would shorten the calving interval in the herd. Culling rate increases with age of the cow as many authors have found that fertility

declines with increasing age after the second or third gestation (Arthur et al, 1989).

ix EARLY EMBRYONIC DEATH

The finding of an early embryonic mortality, possibly as high as 16% was comparable with the results of other researchers who found embryonic loss rates of 14% (Ayalon, 1978) and 15% (Boyd et al, 1969) at 35 days in normal fertile cows. However, the figure was much higher with 29% and 36% in two categories for repeat breeder cows (Ayalon, 1978). There was some controversy about the exact stage at which embryo loss occurred, some claiming that most embryonic loss occurred before Day 8 (Ayalon, 1978) and others after Day 16 (Hawk et al, 1955).

When embryonic death occurs, the animal will return to oestrus. The oestrous interval would depend on the stage at which death occurred and whether or not the uterine disturbance was affecting ovarian dysfunction. In very early embryonic death, that is before day 14 of the cycle, the animal would return to oestrus at a normal cycle length. When normal return to oestrus occurred, it was impossible to determine whether fertilization had failed or whether there was early loss of an embryo. When cows return to oestrus at regular 21 days intervals for more than 3 matings, it is known as a repeat breeder. In beef cows, it has been reported that 10% of cows fell into this category (Maurer and Echternkamp, 1985).

Most of the embryonic loss occurs early in the gestation period resulting in normal return to oestrus (Ayalon, 1981). It had been suggested that many embryonic deaths are due to genetic abnormalities which were unavoidable and often regarded as the normal means

by which unfit genotypes are eliminated (Bishop, 1964). Genetic abnormalities were shown to be only 3% and 14% of early embryos collected from heifers and cows respectively during the first few days of pregnancy (Maurer and Echternkamp, 1985). These figures were too low to account for the rate of embryo loss which actually occurred. Embryonic death occurred following the use of bulls of high fertility while both embryonic loss and fertilisation failure were shown for bulls with low fertility (Bearden et al, 1956).

x UTERINE DISEASE AND ABORTION

Diseases of the uterus are obvious if they result in an abnormal discharge from the genital tract from retained placenta, metritis, and cervicitis. However, there was only one case each of retained placenta and mild uterine discharge observed throughout the period of study. Sporadic incidence of abortion with five (12%) known cases being recorded during the current year (Table 8). The average abortion rate from 1986-1992 was 7.7%. The abortions occurred mainly after the fifth month of pregnancy with no specific relation to season. No specific organisms were identified despite meticulous investigation by the Government laboratory.

Non-specific uterine infections have also been implicated as an important cause of infertility in clinically normal repeat breeding cows (Hartigan et al, 1972). The incidence of uterine bacterial infection in repeat breeding animals reported by other workers varied widely from an infection rate of 80% (Easley et al, 1951) to 10% (De Bois and Van de Akkar, 1957). It might be important, therefore, to determine whether pathogenic bacteria contributed to the cause of infertility by injuring the gametes or the developing conceptus.

In contrast, culture from uterine swabs of the reproductive tracts from the abattoir specimens from animals in farm 2 showed contrasting results. The low incidence of bacteriologically positive uteri was comparable with those found by other investigators (Roberts, 1971) who found that uterine infections were eliminated by the third or fourth service. It was not suggested that the findings from another herd could be applied here nor that the animals from which no pathogenic uterine organism recovered had never had any uterine infection. It could be inferred that if there ever had been an infection, they could have been resolved before the samples were taken. Thus, there was no evidence to suggest that micro-organisms were the primary cause of the infertility.

xi PROGESTERONE ASSAY

It was found by progesterone assay that 19% of the cows were wrongly diagnosed for the presence of a CL. In some cows, a single progesterone sample was taken because the animal was not brought in for examination. In such a case, a low progesterone would only indicate that the cow was not in dioestrus. Concurrently, it would help to confirm the clinical findings where no corpus luteum was palpable. A low progesterone concentration when no corpus luteum was identified was indicative of non-cyclicity or non-luteal phase of cycle. A high progesterone concentration seven days before or after the palpation of ovaries without a corpus luteum was indicative of a non-observed oestrus. In this way cycling cows could be distinguished from noncycling cows or cows that were not detected in oestrus. Confirmation of the diagnosis was obtained when two or more consecutive samples were taken to observe the trend. In this way, it enabled the accuracy

of oestrous detection by rectal palpation to be checked and result forecasted as shown by the progesterone levels of two cows (Graph 9; 10). It was possible for rectal palpation and progesterone assay to become an integral part of herd fertility control programme, expecially confirming diagnosis in doubtful cases.

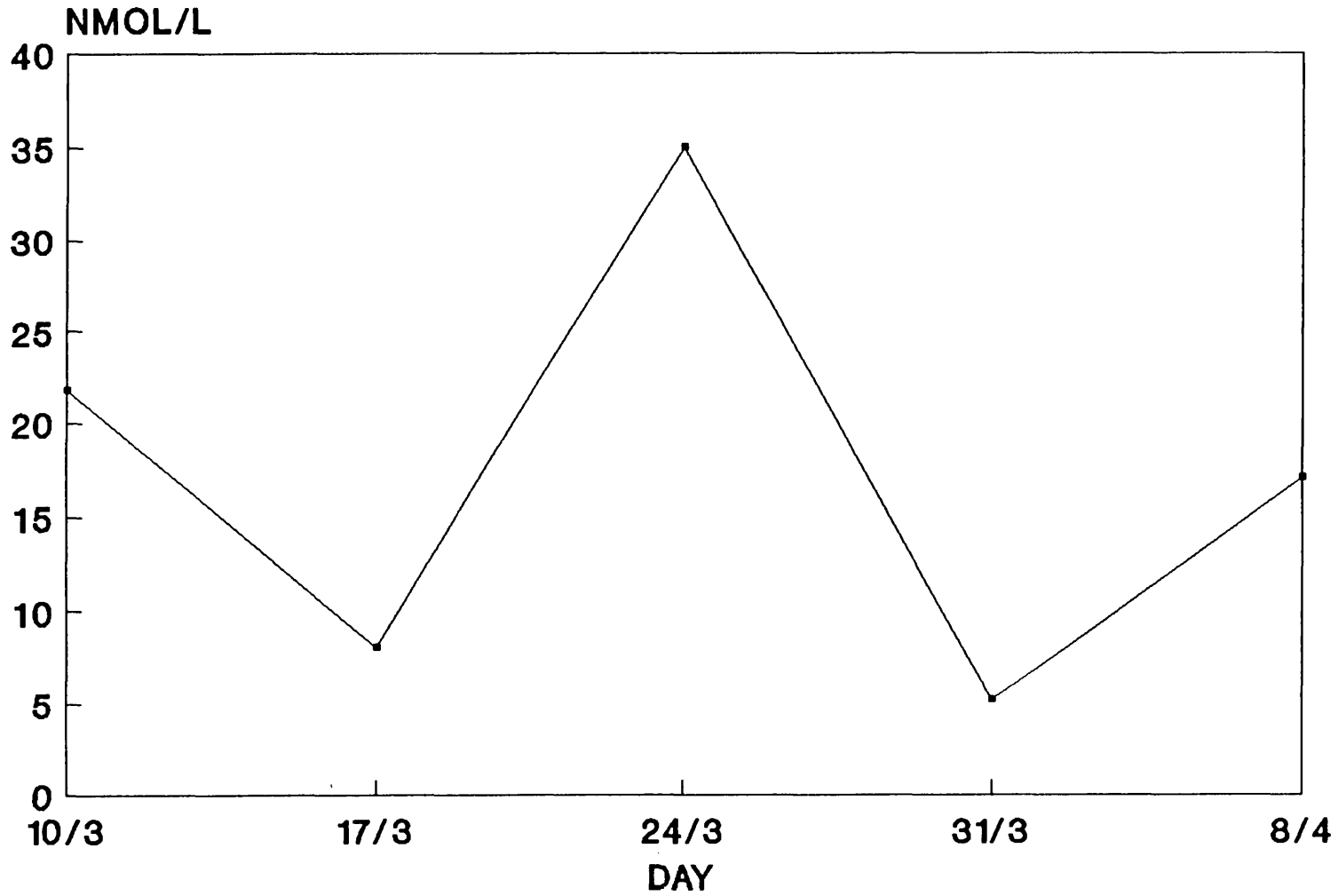
5.2 FARM 2

i GENITAL TRACT ABNORMALITIES

Gross pathological examination of the genital tracts of the sterile cows and heifers from farm 2, revealed that 67% of the tracts had some form of reproductive tract abnormalities (Table 6). Four cows (33%) had minor, clinically non-detectable, ovarian adhesions to the bursa which were acquired and might not had any effect on ovum migration and fertilization (Plate 5). There were 2 cases (17%) of cervical strictures in the heifers which were probably congenital (Table 6). Since there was no noticeable accumulation of fluid in the uterus, it was likely that the cervical strictures were patent to the passage of fluid although not allowing passage of the catheter. In a severe form, this would cause a structural barrier to fertilisation where the sperm were prevented from entering the uterus resulting in fertilitisation failure. This incidence of abnormalities was much higher than reported by others

GRAPH 9 PROGESTERONE VALUES OF JANICE 2

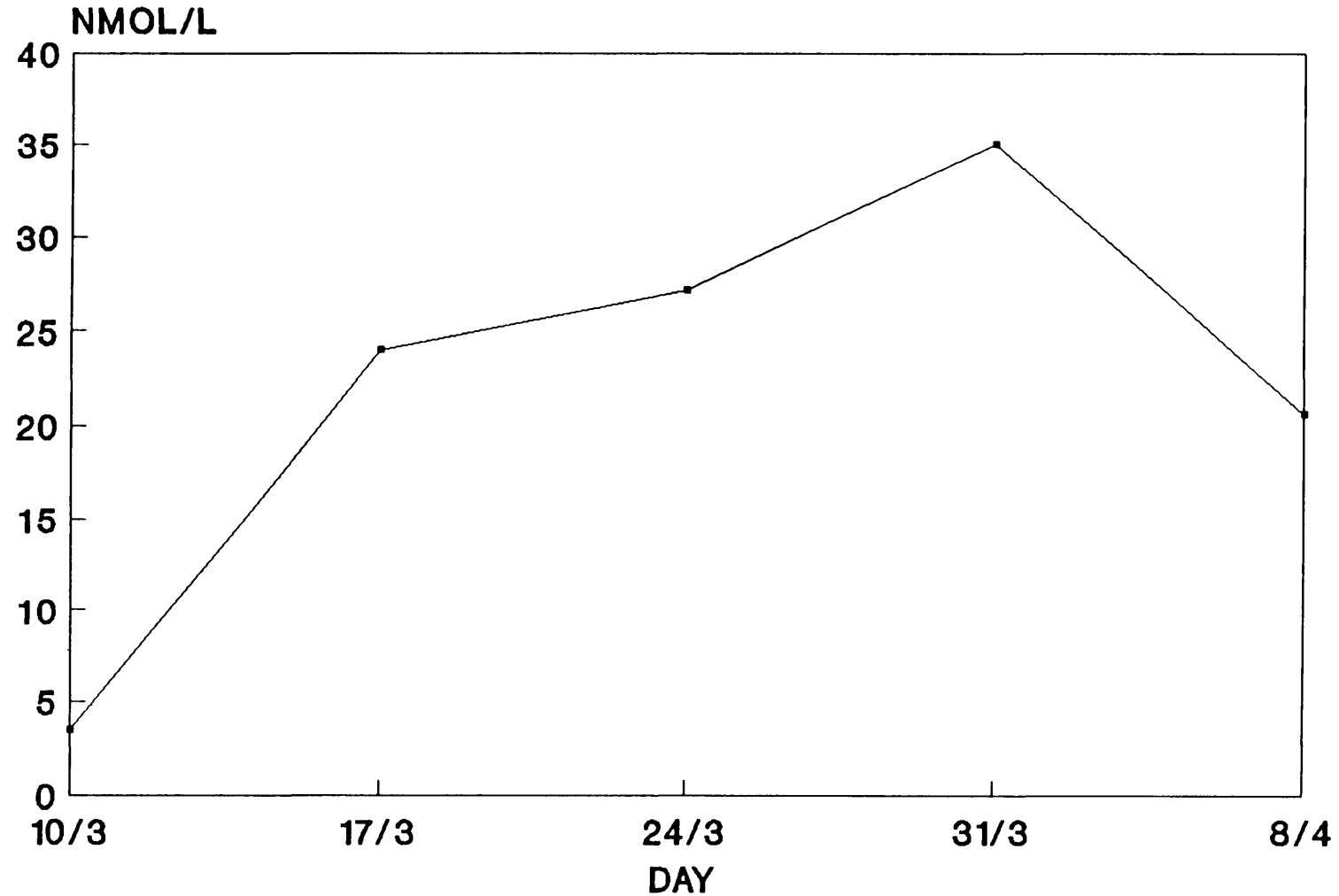
POLLOK FARM 1992



PLASMA PROGESTERONE TAKEN FROM 82 DPP

GRAPH 10 PROGESTERONE VALUES OF KIRSTY2

POLLOK FARM 1992



PLASMA PROGESTERONE TAKEN FROM 82 DPP

who found only 10.6% in cows (Tanabe and Casida, 1949) and 13.5% in heifers (Tanabe and Almquist, 1953). This could be due to cumulative cases of animals which were proven barren and sterile, but were maintained for a long time before being culled. A study of the pedigree of the animals with cervical non-patency showed that there was no relationship between the parents. This could be an important point because it raised the question of prevalence of this problem among the breed in general, rather than specifically among the individuals. Further elucidation of the problem of cervical occlusion was required. It was possible that this abnormality arose as a result of inbreeding within the herd. Hence, it was suggested that an intensive selection programme for breeding bulls and cows free from this defect be carried out in order to overcome this problem. This programme would incorporate culling for cervical strictures on yearling heifers. The fallopian tube occlusion (17%) could be associated with previous endometritis where the infection ascended or originated from the uterus and infected the fallopian tube.

The uteri from cows in farm 2 showed no gross pathological lesions, in agreement with the histological findings. However, these findings differed from a study by Hartigan et al, (1972) which showed 12.5% of the genital tracts obtained from an abattoir had gross lesions and 50% were histologically confirmed for endometritis. This could be due to sampling technique and interpretation of histological slides which required considerable experience of the normal cyclical changes of the endometrium.

5.3 FARM 1 AND 2

i CHROMOSOME ABERRATION

The finding of the 1/29 Robertsonian translocation in the British Highland Cattle breed was of particular interest as this chromosome aberration has not been recognised in this breed even though it has been reported in about 50 other breeds throughout the world. This is, by far, the most common centric fusion found in cattle. The study on the relationship between animals studied indicated that there was a possibility of the chromosome defect originating from the lineage of Prionnsa 4th of Leys as outlined in the hypothesis (Fig 1). The reasons for the hypothesis were as follows:-

On farm 1, four cows and one bull namely Jan, Michelle, Audrey and Ailleag Og 11th of Millerstone and Coinneach of Balblair were found to be positive for the 1/29 Robertsonian translocation.

i. Ten half sibs from the sire of Michelle of Pollok (Fig 2) were found to be free from the translocation. Hence, it could be deduced that the sire was free of the defect as the probability of inheriting the defect was less than 1% ($X^2 = 6.5$, $p < 0.001$). Thus, it would appear to suggest that it was more probable that the dam was a carrier of this defect. More screening of the offspring from this dam was required to establish this fact. However, further screening of the offspring from Angus Og 4th of Glenogle (Fig 1), the sire of Michelle would be desirable, in order to confirm the hypothesis that the chromosomal defect could have been inherited from the great-grand father, Niall of Douneside. This was the common progenitor from whom the probands could have been derived, even though the probability of Angus

Og 4th of Glenogle possessing the defect was negligible as described earlier.

ii. It was established that Jan of Pollok (Fig 3) and her paternal half sib Audrey were carriers of the translocation. Both of these probands had a common father, Coinneach of Balblair. The father of Coinneach of Balblair was Prionssa 4th of Leys, suspected to be the lineage for the inheritance of the translocation (Fig 1). This probability was confirmed and by other probands which were found to share this common sire as outlined in (v) and (vi). Six contemporary half sibs from the sire and one from the dam of Jan in the herd were found to be negative for the defect. However, it was important to note that the dam of Audrey, Maili (Fig 4), was free from translocation. Hence, it could be safely assumed that the translocation was inherited from the common sire Coinneach of Balblair and its previous generation. This bull was traced and its blood sample was obtained after much difficulty. Karyotype analysis confirmed the presence of the translocation in this bull and thus confirmed the above hypothesis. It could be postulated therefore, that the progenitor of this inheritance could derive from his sire Prionnsa 4th of Leys (Fig 1) and from previous generations going back to the great grand father of Coinneach, namely Niall of Douneside.

iii. The sire Coinneach of Balblair (Fig 1), heterozygous for the 1/29 translocation, produced six offspring which were free from the defect and two offsprings which carried the translocation. The one contemporary half sib from the dam of Jan was also found to be negative for the translocation.

iv. Another cow Ailleag Og 11th Millerston (Fig 5) was diagnosed positive for the heterozygous translocation

and required further investigation and testing of offspring and relatives. So far it was difficult to obtain relatives of this cow for the testing programme. Since there was no linkage of any common ancestry to the progenitors Niall of Douneside or Prionnsa 4th of Leys in the hypothesis (Fig 1), it could only be postulated that the progenitors of this translocation were probably more than five generations ago. However, the extend of this defect in the parentage tree remains to be determined. The further back the progenitors of this defect were located in the lineage, the greater will be the extend of the spread of this defect in the breed.

The summary of the inter-relationship of the probands in farm 1 was illustrated in Fig 6.

In farm 2, there were three animals found to have 1/29 Robertsonian translocation, namely Princess Og 46th, Neoinean Og 97th and Princess Ealasaid 38th.

v. Two full sister cows Princess Og 46th (Fig 7) and Neoinean Og 97th (Fig 8) of Achnacloich from farm 2 were positive for the translocation and shared a common sire Prionnsa 4th of Ley. However, six daughters of Prionnsa were diagnosed negative for the defect. Thus, there was a probability of 25% (X^2 =2) that this sire passed this defect to his daughters. Other circumstantial evidence as shown in para (i, ii, iii, iv, and vi) lent support to the hypothesis that the common parentage line from which the defect was assumed to be inherited was from the sire Prionnsa 4th of Leys.

vi. Princess Ealasaid 38th of Achnacloich (Fig 9) was found to be a carrier of the translocation and was the daughter of the sire Jock 25th of Leys, the son of Prionssa 4th of Leys. Two half sibs from Jock 25th of

Leys (Fig 1) were diagnosed free from this defect. Thus, there was a 50% probability that the sire Jock 25th of Leys was a carrier of the defect. This figure was consistent and did not differ significantly from the inheritance of the translocation in Swedish Red and White cattle (Gustavsson, 1969) which corresponded to the normal 1:1 segregation of the translocation observed during meiotic studies in Charolais bulls (Logue and Harvey, 1978). None of the half sibs from the dam were available for screening. Further screening of the siblings from the sire is required to confirm the presence of the translocation in Jock 25th of Leys. Nevertheless, it is reasonable to suggest that the translocation could have come from the lineage of Prionnsa based on the evidence from the probands.

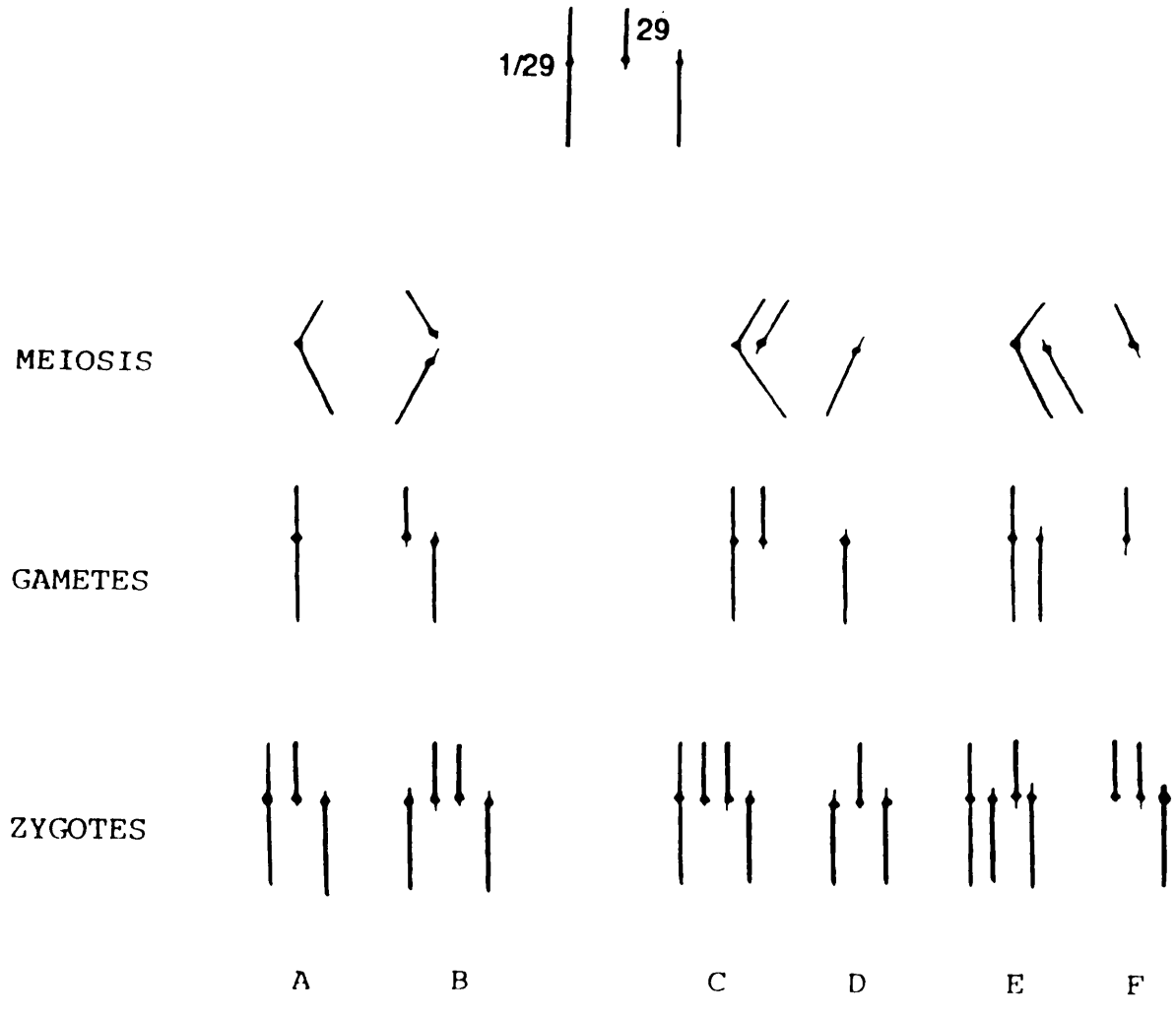
The study of the probands' pedigrees strongly suggested that the expression of this genetic aberration was inherited from the Coinneach of Balbair from the lineage of Prionnsa 4th of Leys and the progenitors would have lived more than five generations ago as shown in para (iv). Thus, it would be reasonable to assume that there are many more animals affected in the breed. This widespread occurrence differed very much from the study by Wilson (1988) in the Friesian breed in which the translocation was confined to a small family locus. The high percentage of incidence in the breed (12%) in a relatively small population could also be due to the small number of animals being screened for the translocation. The incidence was as high as in the Swedish Red and White 14.3% (Gustavsson, 1969) but was lower than the 20.6% in Blonde d'Aquitaine (Queinnec et al, 1974) and the 32.0% in Romagnola breed (Molteni et al, 1977). Despite this, it is likely that there will be many more cases of animals with the translocation in the breed. This is because

the breed is an extremely close breed with a small population.

The effect of centric fusion on reproductive efficiency has been a controversial issue depending on circumstances and magnitude of the problem. In order to understand the occurrence of this problem, the types of gametes produced at meiosis has to be understood (Fig 10). In the centric fusion, two chromosomes were replaced by one and as a result the total number of separate chromosomes in the heterozygous individual will be one less than the normal number. However, this differs from monosomics because it has a complete genome and hence a normal phenotype. In homozygous centric fusion it too had a normal phenotype and a complete genome even though it had two less than the normal number of chromosomes.

In homozygous animals with a centric fusion, there is no problem of early embryonic death because only balanced gametes are being produced resulting in a balanced zygote at fertilisation. However, the situation is different for heterozygous individuals, where three chromosomes have to synapse, forming a trivalent gamete at meiosis. Balanced gametes would result only if the chromosome with translocation disjoined from the other two. Other types of disjunction would lead to unbalanced gametes which would lead to early embryonic death as shown in fig.10. This is because embryos died subsequent to fertilisation in cattle due to trisomic or monosomic zygotes from unbalanced gametes which apparently are capable of fertilisation (King et al, 1981). This was also shown by Gustavsson (1969), who found that in daughters of heterozygous bulls, there was a reduction of about 5% in non-return rate. Similarly, in heterozygous bulls, there was shown a reduction in

FIG 10 TYPES OF GAMETES AND ZYGOTES PRODUCED BY BULL HETEROZYGOUS FOR 1/29 TRANSLOCATION
(Adapted from Gustavsson, 1969)



(A) Balanced carrier; (B) normal; (C) trisomy 29; (D) monosomy 29; (E) trisomy 1; (F) monosomy 1

fertility, although libido, serving ability and volume of ejaculate were normal (Dyrendahl and Gustavsson, 1979).

It has been argued that there was a substantial increase in financial benefits with the increased fertility resulting from the eradication of the 1/29 translocation in cattle (Gustavsson, 1979). The impact on financial benefit would only be noticeable if there was a high frequency of 1/29 centric fusion in the breed. This could be seen in the effect on fertility (Swedish Red and White) with the eradication program when the frequency of the 1/29 centric fusion was at 14.3% (Gustavsson, 1979). In the present study, even though the average frequency was about 12% in the two farms 1 and 2, there was no significant effect of translocation on the herd because the herd size was small, totalling only about 100 breeding animals.

The effect of this aberration on fertility was inconclusive as the sample size used in this study was small. The data of one known carrier animal, Michelle of Pollok showed a prolonged calving interval of 650 days which could be due to embryonic death and was consistent with the effects of translocation on fertility. However, in three other animals with known translocation defects, there was no relationship between the occurrence of translocation and infertility. This was as expected as the incidence of early embryonic death would only be in the region of about 10%. There were no adequate data available in three other animals with the translocation because two were heifers and one was a first calver in farm 2.

Secondly, the financial loss was less because the cost of maintenance of repeat breeders was less in beef

herds and also the Highland breed was economic to maintain. Thirdly, these animals had a long productive life-span as a result it was not obvious when there was a decrease in number of calves born. Fourthly, the problem was relatively new, as this was the first time that the translocation was detected. Last but not least, the financial impact of this problem was minimal as the translocation would not affect the phenotypic make up of the animal. It had been shown that there was no correlation between the translocation and characters such as milk production, body conformation and butter fat (Gustavsson, 1969). The farmer would continue to use this animal which possessed good conformation and genetic qualities, whose values would outweigh the impact of infertility. This could be seen in farm 1, where the sire Coinneach of Balbair was purchased back to be used for breeding because of its good characteristic on body conformation and genetic qualities. Consequently, it was apparent that consideration of the problem of chromosomal aberration would be secondary to the needs of market demand for good body conformation of the animal. Undoubtedly, at the same time, propagation of this defect would continue unconsciously and unabated as there was no regulation controlling its usage. There is a need to carry out a large-scale population survey in order to establish the frequency of occurrence. Further investigation is warranted to confirm the extend of the problem and it would be wise to start eradicating the centric fusion at its early stage to avoid greater loss later on.

6.0 CONCLUSIONS

The British Highland is a very hardy and economical beef breed suitable to a cold harsh climate and which does not require housing, extensive labour nor feed supplement. It has its own distinctive beauty with good conformation, long horns and hair. It might be slow to mature but breeds well into old age with a long productive lifespan of about 20 years. It has been naturally selected for its genetic potential for growth on minimum pastures.

On the whole, the data collected in this study suggested that there was a slight decline in fertility on farm 1. It is a good policy for the farm to set its own targets as reproductive indices depend on the relationship between the inputs and outputs. The study implicated that a few individual cows contributed to the overall decline in performance of the herd as could be seen by the wide range of results obtained. There was a significant variation of result especially on the calving interval which ranged from 320 to 956 days. This became more evident when four cows were still empty at the end of this trial despite being mated repeatedly. A comprehensive study of these four repeat breeders would be useful in determining the aetiology and possibly to provide a solution to the problem. It was difficult to draw any firm conclusions as to the precise cause of decline in fertility on this farm as there was no single contributory factor. However, one could rule out certain aspects and concentrate on the areas which were not covered by the scope of this study in order to determine the probable reason. Non-specific stresses such as handling, subnutrition, suckling, or abnormalities of parturition were factors capable of

producing stress which may be linked to decline in fertility.

Cows are most susceptible to stress at time of mating, around 30-50 days after conception, and at calving. Regrouping of cows at critical times may upset conception or pregnancy with the changes in social structure. Cows which were used to a quiet and constant routine were likely to suffer from problems of reproduction.

Infertility caused by specific disease might be unlikely but nevertheless, it would be worth screening the cows especially with the increase in abortion rate to 12% in the current year. Within the scope of this study, the bulls were not examined except from their breeding records. It was imperative therefore, that the bulls be subjected to scrutiny in order to confirm they were free from all defects and diseases especially Campylobacter. Selective culling of infertile cows and heifers with cervical strictures should be carried out and consideration given to eliminating animals with Robertsonian translocations. However, it has to be accepted that there was no obvious correlation between the drop in fertility and animals with translocation, although the numbers are tiny.

There was no one simple cause of infertility among the herd and as a result, one has to look at the multi-facet ways of improving the fertility through good management and nutrition. This was because the key to better reproductive performance and successful breeding is good management. Since Highland cattle have a higher age at first calving with a longer productive life than many other beef breeds, it was good to introduce artificial insemination in the herd in order to avoid inbreeding. At the same time, genetic improvement could

be hastened and top replacement heifers or bulls could be obtained. One should even contemplate using embryo transfer if required. Crossing of BHC cows with bulls of faster maturing beef breeds like the Aberdeen Angus has been very successful for producing commercial half-breed for meat production, but obviously not in any large scale in pedigree herds such as these two herds.

There is always a certain percentage of infertility, even in a well managed herd. Therefore, it is important to detect them early and to take the necessary action. One quick way of assessing the herd reproductive performance is the maintenance of a good and accurate recording system of the reproductive history of each cow. Since a computer had been purchased on herd 1, it was recommended that record keeping of the herd be computerised. This would enable data to be retrieved easily, action lists to be produced and analysis of the fertility status of the herd made promptly in order to assist in the management decision. Undoubtedly, monitoring of fertility would form an integral part of the program to ensure success. In this way problem cows could be identified, examined and culled to ascertain that targets were achieved. In order to improve fertility to its optimum level, it required the active collaboration of the people involved, namely the herdsman, manager of the farm and the Veterinarian. It was important that fertility targets were agreed upon and a commitment be made to ensure that the system function effectively.

7 APPENDIX

7.1 HYDRALLANTOIS AND CYSTIC OVARIES

While the foregoing work on the infertility study was in progress, two interesting clinical cases were encountered in farm 1, namely one case each of cystic ovarian disease and hydrallantois.

A cow which had calved 29 days earlier was found to have an ovarian follicular cyst of 2.7cm. This was detected by rectal palpation and was confirmed by ultrasound. The appearance of cysts might be normal especially immediately after parturition in dairy cattle, but much less so in beef breeds. Because of the cow's late calving date in the season, treatment was undertaken by administrating 5ml of synthetic GnRH (Receptal, Hoechst) intramuscularly.

Another cow which had previously given birth to two normal calves had an excessive accumulation of fluid in the foetal sacs. The mating date was not recorded but the cow was diagnosed pregnant at about 6 months of gestation. Two months later the cow was found with an excessively large abdomen, but she was bright and ate well. In view of her normal behaviour and proximity to parturition, it was decided to allow gestation to continue. The owner was advised to notify the veterinarian if her condition worsened. However, the cow became anorexic and her condition progressively worsened . A diagnosis of hydrallantois was made by clinical examination. Rectal examination revealed a very distended uterus filled with fluid with palpable cotyledons, non palpable fetus but fremitus could be felt. It was difficult to ascertain whether the fetus was still alive or whether there was any twinning. As the condition worsened, it necessitated action to be

taken before distension become so severe that it would had caused death to the dam. There are four different approaches in overcoming this problem, namely, draining the fluid, caesarean section, induction of parturition by drug or finally, inactivity. However, the plan of action would depend very much on the condition of the dam, the stage of pregnancy and the viability of the calf. Eventually, the treatment chosen was induction of parturition by administration of 20mg dexamethazone (Azium, Schering-Plough Animal Health) intramuscularly.

7.1A RESULTS

i OVARIAN CYST

The cow was found to have a functional corpus luteum by rectal palpation and a progesterone value of 10nmol/l, 7 days after treatment with Receptal. However, there was no palpable reduction in size of the cyst on rectal palpation. Subsequent investigation showed that the cyst had subsided and the cow return to normal oestrus.

ii HYDRALLANTOIS

The dam showed signs of parturition 36 hours after adminstration of dexamethazone. There was fetal dystocia because of breech presentation and aid was given to deliver the calf. A striking feature of this case was that a live calf was delivered with the dam making a speedy recovery. Recovery of the dam was almost immediate compared with caesarean operation which often could take several days to recover (Neal, 1956). In this case the abdomen of the dam assumed a normal appearance within two days. The foetus was not oedematous nor abnormal except that the male calf, which weighed about 25 kg was considered by the farmer

to be premature by about 3 weeks. For the first 3 days there was a poor suckling reflex and the calf was bottle fed. Two weeks later the calf was successfully fostered onto another cow and continued to thrive. After parturition, the placenta was discharged normally within 6 hours. The cow showed oestrus and was bred at 80 days postpartum and was later diagnosed pregnant to the first service.

7.1B DISCUSSION

i OVARIAN CYST

When the ovarian cyst was diagnosed in the cow in June, it was decided to treat it despite the fact that it would probably resolve spontaneously. It is believed that post-partum follicular cysts are frequently self-limiting with spontaneous recovery even if not treated. Treatment was undertaken because it was felt necessary to advance the date of breeding in order to bring the cow into the present mating season. Otherwise, the cow might had been left barren till the next mating season. After treatment with GnRH, there was no noticeable change in physical shape and size of the follicular cyst. However, the plasma progesterone value was found to be 10nmol/l which indicated that a functional CL had formed. This was almost certainly due to the response to GnRH therapy of other follicles. Cystic follicles have been reported during the early postpartum period (Morrow et al., 1969) with a significantly higher incidence occurring in high producing cows (Morrow et al, 1969).

ii HYDRALLANTOIS

There was a risk in treating this case of hydrallantois as there were many other factors which were not

definitive, such as the stage of pregnancy of the dam, the response to treatment and the life of the fetus. In retrospect, it was worth the risk taken, as shown by the satisfactory result. It was surprising that a normal healthy calf was delivered in contrast with other reports that most calves were dead on delivery because of cystic condition of the kidneys (Neal, 1956). Thus, it could be debatable whether the case was indeed a hydrops. Despite this, it was likely that the case presented was a hydrops which was treated at the right time before any deterioration of the fetal kidneys had occurred. This was because the clinical signs exhibited by the dam were typical of a hydrops and were verified by rectal palpation. A healthy calf delivered and surviving until now, indicates that it had normal kidneys. This was consistent with the findings of other authors who suggested that these renal changes were probably due to secondary effects of the hydrallantois (Roberts, 1971). Hence, it could be assumed that in this case, the intervention by induction of parturition was promptly administered at the right time before the allantoic fluid build up could cause damage to the kidneys. This view differed from the common belief that hydrallantois developed because of kidney failure. The dam recovered normally without placental retention, was bred 80 days postpartum and was pregnant to the first service. The high expectancy of maternal recovery following induction of parturition was an advantage over caesarean operation. It had been shown that placental retention was frequent following caesarean (Arthur et al, 1989), probably due to inertia of the uterine musculature consequent to the extreme stretching. This might lead to a protracted convalescence and delayed conception.

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