STUDIES ON UTERINE PATHOLOGY IN REPEAT BREEDING CATTLE

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THESIS

Submitted in partial fulfilment
of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and animal Sciences
Kerala Agricultural University

Department of Animal Reproduction
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

1979

DECLARATION

I hereby declare that this thesis entitled "STUDIES ON UTERINE PATHOLOGY IN REPEAT BREEDING CATTLE" is a bona-fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

K.N. Aravinda Ghosh.

Mannuthy,

3 -12-1979.

CERTIFICATE

Certified that this thesis, entitled "STUDIES ON UTERINE PATHOLOGY IN REPEAT BREEDING CATTLE" is a record of research work done independently by Shri K.N. Aravinda Ghosh under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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ACKNOWLEDGEMENTS

I wish to record my sincere gratitude to Dr. T.N. Bharathan Namboothiripad, Professor (Project Co-ordinator), Cattle and Buffaloes, the Major Adviser, under whose guidance this work was carried out.

I am thankful to Dr. C.P. Neelakanta Iyer, Professor, Department of Animal Reproduction, Dr. A. Rajan, Professor (Project Officer), Department of Pathology and Dr. V. Sudarsanan, Associate Professor, Veterinary Hospital, Mannuthy, members of the Advisory Committee for their valuable suggestions, help and guidance.

I am greatly indebted to Dr. M.S. Nair, Associate Professor, Dr. K. Prabhakaran Nair, Associate Professor and Dr. E. Madhavan, Associate Professor, Department of Animal Reproduction for their valuable help rendered in the conduct of the work and preparation of thesis.

I am grateful to Dr. P.G. Nair, Dean, Faculty of Veterinary and Animal Sciences for the facilities provided for the study.

Grateful acknowledgement is made to Dr. S.P. Suresan Nair, Dr. P.P. Balakrishnan, Dr. K.C. Raghavan and Dr. G. Sukumara Pillai, colleagues for the help rendered to me.

I am grateful to the Kerala Agricultural University for the scholarship awarded to me for my studies.

My thanks are also due to Sri. P.X. Francis for typing the manuscript.

K.N. ARAVINDA GHOSH.

DEDICATED

TO

MY LOVING PARENTS

C O N T E N T S

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INTRODUCTION

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The importance of infertility as a limiting factor for improving livestock production is well recognised. Investigations and surveys carried out during recent decades show clearly that temporary infertility rather than absolute sterility is more commonly encountered in cattle. Of the various conditions leading to impaired fertility in cattle, the problem of repeat breeding assumes paramount importance, posing grave economic threat to farmers all over the world. Even today, failure to breed or repeated return to service for no apparent reason constitutes the biggest problem confronting the dairyman. Inspite of the intensive investigations carried out during the past in different parts of the world, the problem still remains challenging.

Comprehensive review on repeat breeding in cattle has been presented by Casida (1961), Jainuddeen (1965), Hewett (1968), Olds (1969) and Roberts (1971). Research work carried out in the last four decades in various parts of the world outlined the major proportion of the problem, but limited advances have been made in studying the various causes and still less on the diagnosis of causes in individual repeater cows. Obscurity of causes in such cases made the problem challenging to clinicians (Jainuddeen, 1965). Uterine pathology as direct effect or as indirect reflection of ovarian and other disturbances has long

been regarded as the commonest cause. Clinical symptoms of such uterine lesions often cannot be demonstrated by rectal or vaginal examination except in severe cases. Diagnosis on the basis of rectal examination is not reliable and hence the possibility of conclusive diagnosis is also small. In this connection the technique of endometrial biopsy to accurately evaluate the functional competence of the uterus and also to find the pathological changes of the uterus will be of great value.

The present investigation was taken up to evaluate the usefulness of uterine biopsy technique for diagnosis of exact nature of the disease problem in individual repeat breeder cows. It was also envisaged to suggest corrective measures on the basis of the biopsy findings.

REVIEW OF LITERATURE

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One of the most important but poorly understood subjects in bovine infertility is the 'repeat breeder cow'. According to Roberts (1971) repeat breeder cow is one that has normal or nearly normal oestrous cycles, but fails to conceive in spite of its being bred two or more times to a fertile bull. Literature accumulated on the subject during the past has brought to light the vastness of the problem. It is understood to-day that repeat breeding could be due to a multiplicity of causes that vary from herd to herd, cow to cow and from oestrum to oestrum.

In a comprehensive survey Hewett (1968) reported 10 per cent incidence of repeat breeding. It was shown that the incidence varied according to season, it being lowest in spring. The incidence also increased as the herd size increased. Zemjanis (1970) reported an average incidence of 15.1 per cent (ranging from 6.7 to 20.9%). Reports from different parts of the world revealed equal magnitude of the problem (Dessouky and Juma, 1973; Rahman et al., 1975). In India, Khan and Luktuke (1967) observed that the incidence of repeat breeding varied from 14 to 27 per cent. Namboothiripad and Raja (1972) reported that 18.8 per cent of cows were repeat breeders in Kerala. According to Kodagali (1974) the problem was less severe.

Causes for repeat breeding have been theoritically

grouped under two main headings viz., failure of fertilization and early embryonic death, by many workers (Casida, 1961; Salisbury and Vandemark, 1961; Roberts, 1971; Hafez, 1974 and Arthur, 1975). The failure of fertilization and early embryonic death have not been considered as causes in themselves, but only outcome of very many other factors.

Among the factors that impede the normal process of fertilization, ovulatory disturbances and lesions of the salpinx have been considered to be of importance. Van Rensburg and De Vos (1962) described anovulation and delayed ovulation as important causes of repeat breeding. Choudhary et al. (1965), while studying the pattern of oestrous cycle in Haryana heifers, observed high rate (56.88%) of anovulatory oestruses. Sampathkumaran and Iya (1966) recorded 74 cases of ovulatory failure in a total of 235 cows belonging to Red Sindhi, Tharparker and Sahiwal breeds. Rao et al. (1975) observed five cases of salpingeal affection out of 44 repeat breeding cows examined. They concluded that chronic salpingitis with mucosal cysts could obviously interfere with transport of ovum resulting in non-fertilization and repeat breeding. Namboothiripad and Raja (1976) showed that 21.95 per cent of repeat breeding in animals studied by them was due to functional ovulatory disturbances.

Infection of the reproductive organs constituted the most important diagnosed cause of impaired fertility in cattle

and great efforts have, therefore, been made in recent years not only to clarify the routes of these infections but also to establish control measures (Roberts, 1971 and Arthur, 1975). The greatest advance in the understanding of the problem of repeat breeding in recent years has been the demonstration of the role of vibriosis, trichomoniasis and probably brucellosis in causing early embryonic death (Roberts, 1971). Infection of genitalia especially uterus with a variety of organisms has also been shown to be an important cause for early embryonic mortality (Asdell, 1958; Dawson, 1960 and Boyd, 1965). Namboothiripad et al. (1976) isolated the infective organisms from the uteri of repeater cows.

Hafez (1974) held the opinion that prenatal mortality in cattle was mostly due to unfavourable uterine environment resulting in implantation failure. Arthur (1975) expressed that changes in the chemical environment of uterus brought about by deficiency of certain essential metabolites could also cause early embryonic death.

Variations in the natural secretions and luminal fluids of genital tract have been subjected to extensive studies to arrive at reliable diagnostic measures for detecting the actual problem in repeat breeder cows. Investigations on the physical and biochemical properties of oestrual mucus have been carried out during recent years by many workers (Bacic, 1962; Panigrahi,

1965; Luktuke and Roy, 1967; Pattabiraman et al. 1967(a), (b) and (c); Bhosreker, 1973 and Pattabiraman and Venkataswami, 1978). Deo and Roy (1971) observed variation in physical properties of oestrual mucus in repeat breeder cows. They also noticed that the frequencies of agglutination reaction with suspension of whole semen and washed spermatozoa were higher in repeat breeding cows than in normal cows. Namboothiripad (1976) could not derive any positive role of local antibody response against sperms in repeat breeder buffaloes. Giller (1978) found that sperm agglutination titres exceeded 1 in 512 in repeat breeding cows and in cows exhibiting normal fertility the titre did not exceed 1 in 64. The degree in which oestrous mucus was penetrated by spermatozoa was also low in repeater cows.

Studies on the physiochemical attributes of uterine secretions were of help in defining the effect of uterine lesions, but were not of much avail in actual diagnosis of individual cases. Peterson (1965) found that in repeat breeding cows, the oxygen consumption of the spermatozoa in the uterine lavage samples was less when compared to normal breeders. The motility of spermatozoa was also reduced in the uterine secretions from repeat breeder cows. Suga et al. (1975) established differences in the sugar and alcohol concentrations of uterine fluid in sterile and normal cows.

Results of many investigations have shown high rate of

occurrence of uterine lesions in repeat breeder cows. Mild endometritis had long been attributed as a cause of repeat breeding (Brus, 1952; Kampelmachner, 1952). Seitardis and Tsangaris (1973) confirmed endometritis on histological examination of the samples of uterine mucosa in 48 out of 50 repeat breeding cows. Bhosreker (1973) found that in repeat breeding Red Sindhi, Sahiwal, Tharparker cross bred females, endometritis was a common feature. Cupps (1973) detected uterine lesions in 55 per cent of repeat breeder cows. The main lesions observed were perivascular, periglandular and subsurface infiltration of cells. He opined that mild uterine infections resulted in periglandular fibrosis, when allowed to continue and this would jeopardise the functional competence of the endometrial glands, thereby posing the threat of incurable infertility in affected animals. Uterine infection with non-specific organisms was reported to be the important cause of repeat breeding (Namboothiripad and Raja, 1976).

Clinical symptoms of endometritis often cannot be demonstrated by rectal or vaginal examinations. Diagnosis on the basis of the rectal examination is not reliable and hence the technique of endometrial biopsy to accurately evaluate the function of the uterus was developed. Endometrial biopsies were valuable aids for obtaining information on endometrium (Hellway, 1971). Extensive use and adaptation of the endometrial biopsy has been done in human medicine (Noyes, 1956 and

Nugent, 1963), both for experimental and diagnostic use, but literature on the use of endometrial biopsy in veterinary medicine is scanty. The devices for obtaining endometrial samples for bacteriological and histological studies vary from a glass speculum and glass pipette (Hatch et al., 1949 and Easley et al., 1951) or two telescoping metal tubes (Frank and Bryner, 1952 and Gunter et al., 1955) to three telescoping metal tubes used with a spreading vaginal speculum, forceps to bring the cervix into position and disinfection of the cervical os (Kampelmachner, 1954).

Several efficient biopsy instruments have been designed, notably, Thye's end cutter (Kaay, 1950), Folmer-Nielson's two tube model (Rasbeck, 1950), a modification of the Folmer-Nielson model by Donker (1952) and a cutting tong used by Skjerven (1956) for taking endometrial tissue for pathological studies. Brus (1952) studied the various aspects of endometrial biopsy and concluded that majority of reproductive disorders were detectable by the technique. He also reported that 13 out of 25 repeat breeder cows conceived as a result of insemination immediately after the biopsy. Kampelmachner (1952) found 18 out of 91 temporarily sterile animals to be affected with endometritis on histological examination of endometrial biopsy samples using Folmer-Nielson biopsy instrument. The instrument used by Skjervan (1956) consisted of a universal handle, an

interchangeable tube and a cutting tip. He obtained a conception rate of 57.3 per cent for animals exposed to this technique. Hardenbrook (1958) collected uterine samples using a biopsy instrument which was a modification of one deviced by Nielson (1949).

Dozsa et al. (1960) developed a new technique for taking endometrial biopsies. The instrument was later employed for studying the histological changes in the uterine mucosa of experimentally vibrio-infected cows (Dozsa et al. 1962). They found lymphocytosis of endometrium as the main lesion. Dozsa and Olson (1964) utilised this technique for gauging the efficacy of antibiotics in vibrio-infections. The instrument developed by Minocha et al. (1964) consisted of four telescoping metal tubes which helped in taking both histologic and bacteriologic samples. Histological changes in the endometrium of intact animals were studied by a biopsy technique on 17 normal cows without any deleterious effect (Johnson, 1965). McQueen (1967) obtained endometrial biopsy samples using a modified Folmer-Nielson cannula-trocar instrument from six cows in a two week period after insemination without seriously altering the conception rate for that service.

Larson et al. (1970) studied the glycogen metabolism in the bovine endometrium by collecting endometrial biopsies from the caudal portions of the uterine cornua. Endometrial biopsies

were made use of for the study of the ultrastructure of bovine endometrium by many workers (Stinson et al., 1962; Marinov and Lovell, 1968). Muursepp (1972) collected biopsy samples of endometrium from 113 clinically normal cows on the day after artificial insemination. He established a significant correlation of -0.36 between conception rate and the degree of lymphocytic infiltration of the endometrium. Afansjevs (1972) examined histologically endometrial biopsy samples from 427 infertile cows, 56 per cent of which had cystic hyperplastic endometritis, non cystic endometrial hyperplasia, endometritis or a diffuse infiltration of inflammatory cells with desquamation of the endometrial epithelium and vascular changes. It was found that, while infertility in cows with normal endometrium never exceeded seven per cent, 37 per cent of cows with various forms of endometritis due to non-specific infections remained sterile. Zafracas (1974) observed cellular infiltration of the endometrial stroma with round cells, neutrophils and eosinophils, lymphatic aggregations and areas of necrosis in endometrial biopsies from 117 infertile cows.

A new instrument was developed by Aria (1976) which consisted of a rotatable cannula, with a fenestrated bulb at the tip which removes fragments of endometrium. Biopsy specimens were taken from 50 cows and the process of biopsy was well tolerated and caused minimal haemorrhage.

From India, only limited reports have appeared on uterine changes of repeat breeder cows and still less on uterine biopsy of repeat breeders. Bhandari (1973) conducted endometrial biopsy in 102 Sahiwal cows and observed that 13.8 per cent biopsies showed normal endometrium and 86.2 per cent showed endometritis.

MATERIAL AND METHODS

MATERIAL AND METHODS

Cross-bred Jersey and Brown Swiss cows belonging to the University Livestock Farm, Mannuthy were utilized for the purpose of study. All animals in this herd were under regular reproductive health control programme. The cows which had failed to conceive even after three or more inseminations with the semen of fertile bulls were considered as repeaters. Twenty six repeat breeding cows which showed no obvious clinical abnormalities of the genital tract and had normal or nearly normal oestrous cycle formed the experimental animals. Four cows which settled at first insemination were also utilized in the same manner as in the experimental animals for comparison.

Uterine biopsy was taken during the early part of oestrum say, four to six hours after beginning of the heat from the 26 repeat breeding cows and four healthy controls. Every cow was inseminated after 8 to 12 hours of biopsy with good quality semen and treated with one gram streptomycin and 8 lakh units penicillin in 30 ml sterile distilled water after 12 to 24 hours of artificial insemination (Ramadas et al., 1978). All the animals were watched for bleeding or any other abnormalities subsequently and were regularly checked for recurrent heat and pregnancy.

Biopsy instrument

The instrument used for collection of endometrial biopsy was a modification of one designed by Minocha et al. (1964). The instrument essentially consisted of four telescoping units (A, B, C and D) accurately fitted together with parts readily interchangeable for taking endometrial biopsy samples (Fig. 1). In all the four units the inner diameter of the outer unit and the outer diameter of the inner unit were almost same so that the units fitted tightly (Fig. 2).

Unit A: It was a stainless steel tube 30.5 cm long and 12 mm diameter which acted as the vaginal speculum. This was made so that the unit B fitted properly inside the tube A. The basal end of the unit A was split so that the whole set could be set up together to a knurled collet.

Unit B: This was a stainless steel tube 50.5 cm long and 7.3 mm outside diameter. In operation, the tube B was inserted to about the middle of the cervix.

Unit C: It was a stainless steel tube of 57.5 cm long and 5.9 mm outside diameter. This was fitted inside the tube B before operation. A handle about 5 cm long was fitted around one end of this tube and welded to it. In addition, one cm below from the tip of the tube C had a window having 0.5 cm length and 0.5 cm width into which the endometrium could be

pressed during operation.

Unit D: It consisted of a stainless steel rod 64.5 cm long and 4.3 mm outside diameter. This acted as the inner most unit and the cutter. The tip of this rod D was made sharp so that the endometrium could be cut off easily. The biopsy instrument was sterilized before use.

Procedure of taking the biopsy sample

Before the samples were collected, the external genitalia of the cows were washed with soap and water and then disinfected with 1 : 1000 potassium permangnate lotion. The instrument was inserted into the vagina and guided by the hand in the rectum, into the cervical os. Then the collet was loosened and tube B was advanced to about the middle of the cervical canal or at least past the first cervical ring, and the collet was again tightened. The tube C was advanced through the cervical canal into the uterine lumen at the position of the uterine body. The endometrium was pressed firmly into the hole in C, then the cutter D advanced with a slight rotatory action and the bit of endometrium protruding into C was sheared cleanly from its source. The tube C was then withdrawn into tube B, the tube B was withdrawn into tube A and the entire assembly withdrawn from the vagina.

Processing

Immediately after taking biopsy, the endometrial piece was transferred to physiological saline to remove the clots and mucus. The clean tissues were transferred to Bouin's fluid for a period of 6 to 12 hours for fixation. They were then transferred to changes of 70 per cent isopropyl alcohol till the colour of the Bouin's fluid disappeared. For dehydration and clearing, the following steps were followed after standardisation with materials collected from slaughter house.

50% Alcohol	1 change	10 mts.
70% Alcohol	1 change	10 mts.
80% Alcohol	1 change	10 mts.
90% Alcohol	1 change	10 mts.
95% Alcohol	1 change	10 mts.
100% Alcohol	2 changes	10 mts. each
Alcohol xylene mixture	1 change	10 mts.
Xylene	2 changes	2 to 3 mts. each

Embedding was done with paraffin wax having melting point of 59 to 60°C. When possible, the fragments of endometrium were embedded in the paraffin so as to orient the mucosal surface perpendicular to the plane at which the block was sectioned. The sections of size of 3 to 5 microns were stained by haematoxylin-eosin method.

Seven out of 17 animals which failed to conceive even after biopsy and treatment with antibiotics were slaughtered. Their complete genitalia were collected immediately after slaughter and the lesions in various parts were studied in detail by gross as well as microscopic examination by standard histopathological techniques. The patency of the fallopian tube was tested by tubo-inflation with 0.5 per cent methylene blue solution (Namboothiripad, 1976).

RESULTS

RESULTS

The general breeding history and clinical findings of the 26 experimental and four control animals are given in Tables 1 and 2 respectively. The cycle length of repeat breeders ranged from 17 to 33 days. The oestrual discharge was thick in consistency in 16 out of 26 repeaters (61.54%) and out of these three (18.75%) only conceived. In two animals, however, turbidity of mucus was also apparent. It could be seen from Table 1 that 10 out of 26 repeaters had post-partum complications in the previous breeding.

Biopsy

Uterine biopsies were taken from all the cows during the early part of heat. The animals showed little evidence of pain while taking the samples. In two cows, the oestrual discharge was slightly blood tinged on the following day of biopsy. In one cow, bleeding was observed which amounted to about 5 ml. In two animals which were slaughtered 24 hours after taking biopsy, it was difficult to discover the wound in the endometrium. However, a small mark at the site of biopsy was observed on close examination. Aparently the biopsy operation had no permanent illeffect on the endometrium. The size of the endometrial piece obtained in biopsies varied from 3 to 4 Cu mm. It was approximately 1.5 mm in depth and, therefore, showed the tubular portions

of the endometrial glands clearly. The biopsies included both caruncular and intercaruncular areas of the endometrium.

The biopsies obtained with the instrument generally appeared to yield a reliable picture of the structure of the uterine mucous membrane. Usually parts of the deeper layer of the endometrium were obtained, but only rarely did a part of the muscularis occur. Out of 26 repeat breeding cows, the biopsies showed evidence of uterine changes in 14 cases and 12 were apparently normal. The uterine biopsy specimens of four control animals which had conceived in the first heat itself were used for comparison. The uterine lesions were recorded and interpreted in the light of breeding history and gross findings.

As uterine biopsies were surgically derived, some amount of vascular changes unrelated to spontaneous physiological and or pathologic origin were observed in all specimens. Lesions of hyperemia, accumulation of fibrin and haemorrhage were of this nature (Fig. 3). It was also common to see intussuspection of glands, particularly around the margin of the sample, as a result of compression occurring during the procurement of the sample. These lesions were considered as artifacts.

Lesions

The various pathological changes in the endometrium are briefed in Table 3. The epithelial lining of the endometrium was intact in most cases, but was partially denuded in few cases. The epithelium consisted of long columnar cells with clear nuclei. In one case there was cytoplasmic vacuolation in the luminal epithelial cells.

Beneath the lining epithelium the endometrial glands showed varying degree of pathological changes. In 20 cases functional activity of the glands with secretions inside the lumen could be seen. Specimens from six animals showed inactive glands without secretions in the lumen. Fourteen out of 26 animals showed lesions of inflammatory nature in the biopsy samples. Infiltration of inflammatory cells at various sites in varying degree constituted the predominant change (Fig. 4). The cells noticed predominantly were lymphocytes, plasmacells, macrophages, eosinophils and polymorphonuclear cells. The cells were occasionally found in the uterine lumen of a biopsy specimen; but more commonly in the luminal epithelium and within the lumen of the glands (Fig. 5). Sometimes they occurred as foci of widespread distribution. In addition to being in the strona, the cell reactions occurred as para - or periglandular collections (Fig. 6) as well as in association with vessels, particularly

veins. In one case there were clumps of bacterial organisms within and outside the glands (Fig. 7). In one case the lymphatic vessels were packed with lymphocytes which was associated with inflammatory changes in other areas also. There was presence of macrophages along with mononuclear cells in one case. Plasmacells were mostly observed below the luminal epithelium. In two cases eosinophils with other inflammatory cells were seen in the lamina propria.

In addition, periglandular fibrosis was observed in three cases examined (Fig. 8). Sometimes the fibrosis occurred in the submucosa also, frequently associated with the presence of inflammatory cells. Periglandular fibrosis of variable degree consisted of closely packed, concentrically arranged, spindle shaped cells with a slender nucleus. In some cases initiation of fibrosis was marked by the loss of randomization of cells and nuclei of lamina propria. This was most readily seen around the glands. Fibrotic changes occurred in isolated glands usually. It was observed that the periglandular fibrosis was observed in the aged repeat breeding cows, which had the history of four or more calvings. The three animals which showed fibrosis failed to conceive even after biopsy and treatment.

Cystic dilatation of uterine glands was observed in three cases (Fig. 9). At the centre of the lumen of the

glands inspissated secretions were seen. This material was eosinophilic and was hyaline. Epithelial cells of the cystic glands in one case showed atropic changes. The cystic glands had wide branched lumina lined by low cuboidal type epithelium. Out of three animals with cystic glands only one conceived.

In one case, an active endometrium with scattered individual glands exhibiting epithelial hypertrophy was observed (Fig. 10). Focal endometrial atrophy was found in two cases (Fig. 11). In these cases also the animals failed to conceive even after biopsy and treatment. Biopsy specimen from one repeat breeder cow showed focal glandular hyperplasia. In this case, there was cystic dilatation of the glands also. Nesting of endometrial glands was found in another specimen (Fig. 12) with no other major changes in the endometrium. The animal conceived in the subsequent insemination. In two cases, there occurred sclerosis and stromal hyalinization.

Interpretation of histologic findings

In order to evaluate the effect of the various pathologic changes on the repeat breeding problem in experimental cows, endometrial lesions were classified into three groups.

Group I: This group consisted of animals which were showing endometrium without any significant pathological changes. The endometrium was neither hypoplastic nor atrophic and was

showing functional uterine glands without any signs of inflammation. The presence of few mononuclear cells was considered normal. The changes in the endometrium of this group were considered not to interfere with its ability to support a fetus to term. This group presented similar microscopic picture as the normal ones. Very mild (occasional) monocytic infiltration with oedema and hyperaemia of submucosa and functional glands were the histologic picture of the four normal cows which conceived at first service (Fig. 13).

Group II: This type of endometrium contained more extensive or more severe inflammatory changes. Mild to moderate, diffuse or scattered infiltration with mononuclear cells in the glandular layer and submucosa were the characteristic features in this group. It appeared that such inflammatory changes and slight fibrotic changes were sufficiently severe to interfere with the ability of the endometrium to sustain pregnancy.

Group III: Widespread periglandular fibrosis, severe cystic dilatation, atrophic glands and severe inflammatory changes were grouped under this category. Inflammatory changes were so widespread and severe that they contributed an essentially continuous infiltration. Moderate to heavy infiltration of plasmacells appeared to be grounds for an even graver prognosis than lymphocytic infiltration.

The conception rate in the experimental animals grouped according to the severity of pathological changes are furnished in Table 5. There was a marked difference in conception rate between the groups. In group I, which consisted of animals showing almost normal endometrium, conception rate of 58.33 per cent (7 out of 12) was observed. But group II which consisted of animals showing more inflammatory changes had a conception of 25 per cent (2 out of 8). In group III, out of six animals none were pregnant. The overall conception rate in the experimental animals was 34.62 per cent.

Observations on slaughtered animals

In order to corroborate the biopsy findings, seven out of 17 repeat breeders, which failed to conceive even after biopsy and repeated treatments were slaughtered and their genitalia were examined in detail. The observations on gross examination of genitalia are presented in Table 6.

In all the animals, the ovaries appeared normal with signs of oestrual activity. Corpus luteum of varying size were observed in all the cases. In one case there were thin fibrous strands between the ovarian surface and bursa. No other abnormality could be detected in any case.

Salpinx in most cases appeared normal on gross

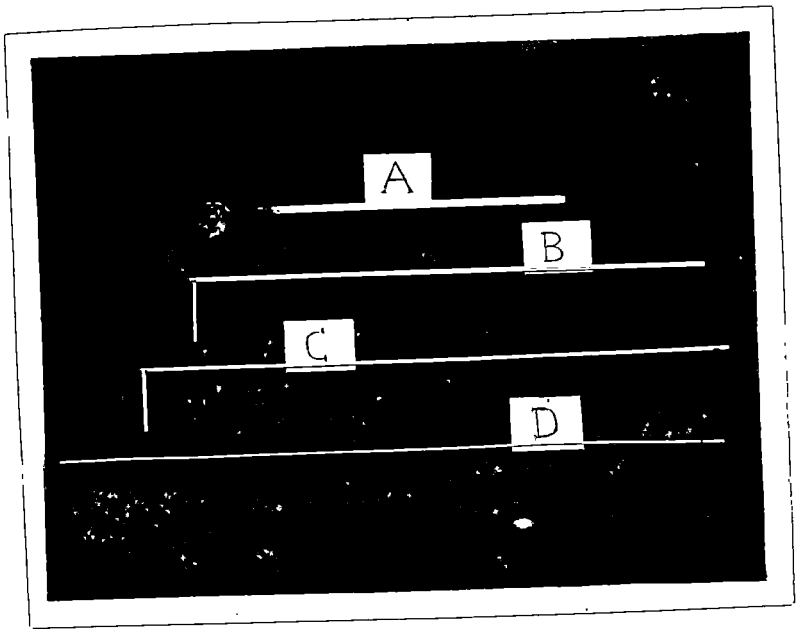
examination. However, in two cases there was thickening of the fallopian tube and unilateral blockage of these tubes was noticed on hydroinsufflation test. Histopathological examination of sections of salpinx revealed vacuolation and degeneration of epithelial cells in one case and lymphocytic cells within the lumen in the other (Fig. 14).

The uterus appeared normal on gross examination in most cases. Congested areas and mucopurulent coating in the endometrium was found in two cases. In one case, the uterus contained yellowish pus with a small dead embryo seen towards the middle of the left horn. Cervix and vagina appeared normal in all cases except in one in which the cervix was oedematous and swollen with slight mucopurulent coating.

Sections of the uterus revealed inflammatory changes of varying degree in all cases on histological examination (Fig. 15). Periglandular and perivascular infiltration with mononuclear cells was a common finding. Moderate periglandular fibrosis was observed in two cases. Focal cystic dilatation with desquamated epithelial cells within the lumen was observed in few cases. Stromal hyalinization was also observed in few cases.

Out of seven animals slaughtered, sections of uterus of six cows were comparable with that of biopsy lesions. In one case, the uterus from the slaughtered animal showed

infiltrating cells mostly lymphocytes and plasmacells in the endometrium but in the biopsy the infiltrating cells were few.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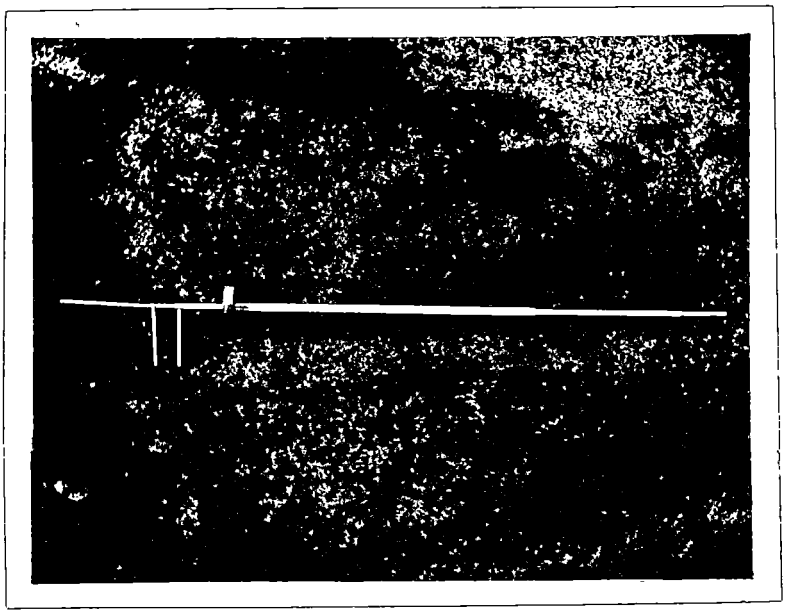

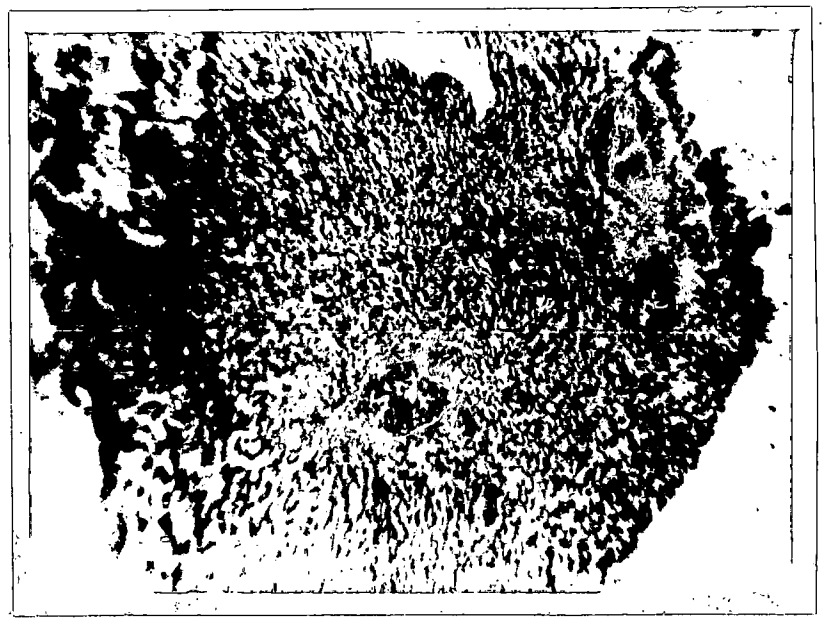

Table 1. Clinical observations of experimental animals.

Sl. No.	Cow No.	Age (years)	No. of calvings	Post-partum complications	No. of ineffective services	Average periodicity of return to service (days)	Clinical abnormalities
1	634	6	3	Normal	6	26	Thick oestrual discharge
2	741	4	2	Retained placenta	7	27	Thin oestrual discharge
3	609	6	3	Normal	7	28	Slight flakes of pus in oestrual discharge
4	431	5	2	Dystokia	4	26	Normal
5	503	4	1	Retained placenta	5	27	Thick oestrual discharge
6	108	8	3	Normal	5	26	Normal
7	475	5	2	Dystokia	6	28	Thick oestrual discharge
8	K.595	7	3	Normal	8	19	Normal
9	611	5	2	Normal	7	25	Thick discharge
10	613	5	2	Retained placenta	7	24	Normal
11	624	5	2	Normal	8	22	Thick discharge
12	636	5	2	Normal	7	30	Discharge thick and cloudy
13	K.641	5	2	Normal	6	29	Normal
14	K.813	7	3	Dystokia	8	28	Thick discharge

(Table 1 contd.....)

Sl. No.	Cow No.	Age (years)	No. of calvings	Post-partum complications	No. of ineffective services	Average periodicity of return to service (days)	Clinical abnormalities
15	365	7	4	Normal	5	24	Thick oestrual discharge
16	667	3	1	Retained placenta	5	22	Thick oestrual discharge
17	471	4	1	Retained placenta	6	28	Normal
18	618	5	3	Normal	6	23	Thick discharge
19	K.644	7	3	Normal	6	28	Normal
20	629	4	2	Normal	5	29	Discharge thick and cloudy
21	429	5	2	Retained placenta	7	28	Thick and scandy discharge
22	817	3	1	Normal	5	26	Thick discharge
23	T.616	3	1	Normal	8	28	Thick discharge
24	C.57	5	2	Normal	7	28	Normal
25	513	3	1	Normal	4	24	Normal
26	U.108	4	1	Retained placenta	6	26	Thick discharge

(Table 1 concld.)

Table 2. Clinical observations of control animals.

Sl. No.	Cow No.	Age (years)	No. of calvings	Post-partum complications	No. of ineffective services	Average periodicity of return to service (days)	Clinical abnormalities
1	962	7	4	Normal	1	20	Normal. Clear, stringy oestrual discharge
2	559	3	1	Normal	1	21	Clear, stringy oestrual discharge
3	415	4	2	Normal	1	23	Normal
4	512	3	1	Normal	1	21	Normal

Table 3. Uterine biopsy of experimental animals.

Sl.No.	Cow No.	Post-biopsy observations	Post-biopsy fertility	Biopsy lesions			Group number
				Epithelium	Glands	Submucosa	
1	634	Slight blood tinged oestrual discharge	Not conceived	Epithelium intact - long columnar cells	Few secretary glands - periglandular fibrosis (moderate)	Mononuclear cell infiltration (moderate)	III
2	741	No visible change	-do-	Denuded	Glandular dilatation - mild focal mononuclear infiltration	Focal and diffuse mononuclear infiltration	II
3	609	-do-	-do-	Denuded	Glands inactive - few in number - periglandular fibrosis	Mononuclear infiltration with lymphocytes and plasmacells	III
4	431	Slight bleeding (nearly 5 ml)	Conceived	Normal	Most glands functional - slight dilatation of glands	Very few infiltrating cells	I
5	503	No visible change	Not conceived	Normal	Few, inactive glands - mild periglandular fibrosis	Mononuclear infiltration	II
6	108	-do-	-do-	Normal	Few mononuclear infiltration	Normal	I
7	475	-do-	-do-	Normal	Focal cystic dilatation of glands	Stromal hyalinization and sclerosis - moderate infiltrating cells	II

(Table 3 contd.....)

Sl.No.	Cow No.	Post-biopsy observations	Post-biopsy fertility	Biopsy lesions			Group number
				Epithelium	Glands	Submucosa	
8	K.595	Slight blood tinged oestrual discharge	Conceived	Denuded	Functional uterine glands	Mononuclear infiltration mostly lymphocytes - neutrophils and few eosinophils seen	II
9	611	No visible change	Not conceived	Normal	Glands sparse and atrophic	Mononuclear infiltration (severe) - clumps of bacteria seen	III
10	613	-do-	Conceived	Normal	Glands functional	No infiltrating cells	I
11	624	-do-	-do-	Normal	Glands functional - nesting of glands	Few infiltrating cells	I
12	636	-do-	Not conceived	Normal	Glands inactive - mononuclear cells within and outside	Mononuclear infiltration (severe) spreading to interior	III
13	K.641	-do-	Conceived	Normal	Functional glands - mild infiltrating cells	Mononuclear infiltration, mostly lymphocytes	II
14	K.813	Slight blood tinged discharge	Not conceived	Normal	Glands inactive - cystic dilatation	Moderate lymphocytic infiltration	II
15	365	No visible change	Conceived	Normal	Glands functional	No infiltrating cells	I
16	667	-do-	Not conceived	Normal	Glands functional	Few lymphocytes seen	I

(Table 3 contd.....)

Sl.No.	Cow No.	Post-biopsy observations	Post-biopsy fertility	Biopsy lesions			Group number
				Epithelium	Glands	Submucosa	
17	471	No visible change	Not conceived	Denuded	Glands functional	No infiltrating cells	I
18	618	-do-	-do-	Normal	Focal nodular glandular hyperplasia - cystic dilatation - desquamated epithelial cells within the luman	Stromal oedema - severe lymphocytic infiltration	III
19	K.644	-do-	-do-	Normal	Functional glands	Normal	I
20	629	-do-	-do-	Normal	Glands atrophic - non-functional	Moderate mononuclear infiltration	II
21	429	-do-	-do-	Normal	Functional glands	Normal	I
22	817	-do-	Conceived	Normal	Glands functional	Few mononuclear infiltrating cells	I
23	T.616	-do-	Not conceived	Denuded	Glands showing degenerative changes with necrotic mass within lumen	Moderate diffuse cellular infiltration	III
24	C.57	-do-	Conceived	Normal	Functional glands	Normal	I
25	513	-do-	Conceived	Normal	Functional glands	Few infiltrating cells	I
26	U.108	-do-	Not conceived	Denuded	Focally periglandular mononuclear infiltration	Diffuse cellular infiltration with mononuclear cells	II

(Table 3 concld.)

Table 4. Uterine biopsy of control animals.

Sl.No.	Cow No.	Post-biopsy observations	Post-biopsy fertility	Biopsy lesions			Group number
				Epithelium	Glands	Submucosa	
1	962	Slight blood tinged discharge	Conceived	Normal	Glands numerous, functional	Normal	I
2	559	No visible change	Conceived	Normal	Glands functional	Few infiltrating cells	I
3	415	No visible change	Conceived	Normal	Glands functional	Stromal oedema - no infiltrating cells	I
4	512	No visible change	Conceived	Normal	Glands functional	Normal	I

Table 5. Conception rate in experimental animals.

Category	No. of animals	Percentage	No. of animals conceived	Percentage
Group I	12	46.15	7	58.33
Group II	8	30.77	2	25.00
Group III	6	23.08	Nil	-
Total	26		9	34.62

Table 6. Gross observations on genitalia of slaughtered animals.

Sl. No.	Cow No.	Ovary	Salpinx	Uterus	Cervix, vagina and vulva
1	741	L.O - RgCL R.O - GF Fibrous strands between L.O. and bursa	NAD	Slight congested areas in the right horn	NAD
2	634	L.O - RgCL and a small DF R.O - Small DF	NAD	Mucopurulent coating on the endometrium	NAD
3	475	R.O - DF L.O - RgCL	NAD	Congested areas in the left horn	NAD
4	618	L.O - RgCL and a GF R.O - DF	NAD	Yellowish pus in both horns with decaying embryo in the left horn	NAD

(Table 6 contd.....)

Sl. No.	Cow No.	Ovary	Salpinx	Uterus	Cervix, vagina and vulva
5	629	R.O - CL and DF L.O - Smooth	NAD	NAD	NAD
6	429	R.O - RgCL L.O - GF	Slight thickening and blockage of left tube	NAD	NAD
7	U.108	R.O - GF L.O - RgCL and DF	Slight thickening and blockage of right fallopian tube	NAD	Oedema, congestion and mucopurulent coating of cervix

(Table 6 concld.)

LO - Left ovary
RO - Right ovary
CL - Corpus luteum
RgCL - Regressing Corpus luteum
GF - Graafian follicle
DF - Developing follicle
NAD - No abnormality detected

Table 7. Histologic observations on genitalia of slaughtered animals.

Sl. No.	Cow No.	Ovary	Salpinx	Uterus	Cervix, vagina and vulva
1	741	NAD	NAD	Denudation of epithelium - marked glandular dilatation - hypertrophy - periglandular fibrosis (moderate degree). Focal periglandular and perivascular mononuclear infiltration - vacuolation of glandular lining cells - nesting of glands.	NAD
2	634	NAD	NAD	Epithelium intact, normal - glandular dilatation and focal mononuclear infiltration in the submucosa.	NAD
3	475	NAD	NAD	Epithelium intact, normal - focal cystic dilatation of glands - submucosa showed diffuse infiltration with lymphocytes and plasmacells - stromal hyalinization and sclerosis.	NAD
4	618	NAD	NAD	Lining epithelium intact and normal - cystic dilatation of glands - focal nodular glandular hyperplasia - diffuse infiltration with lymphocytes.	NAD

(Table 7 contd.....)

Sl. No.	Cow No.	Ovary	Salpinx	Uterus	Cervix, vagina and vulva
5	629	NAD	Dilated lumen - inflammatory cells, mostly lymphocytes.	Epithelium normal - glands inactive - few in number - mononuclear infiltration in the submucosa.	NAD
6	429	NAD	Dilated lumen - vacuolation and degeneration of epithelial cells.	Epithelium normal - glands inactive - few in number - moderate mononuclear infiltration in the submucosa.	NAD
7	U.108	NAD	Tall lining cells - lumen contained eosinophilic exhudate - mild lymphocytic infiltration.	Moderate to severe diffuse cellular infiltration (lymphocytes, macrophages and plasmacells) in the subepithelium - focally periglandular - stromal hyalinization.	Cervicitis observed. Infiltrating cells, mostly mononuclear cells were seen

NAD - No abnormality detected.

(Table 7 concld.)

DISCUSSION

DISCUSSION

The present investigation was carried out to study the uterine pathology in repeat breeder cows, with the object to arrive at diagnostic and curative measures for tackling the problem. The studies were carried out on 26 cross-bred cows of the University Livestock Farm, Mannuthy. All the experimental animals selected were apparently healthy and normal except for failure to conceive even after three or more inseminations with semen from fertile bulls. It could be seen from Table 1 that all the experimental animals had normal or nearly normal oestrus and oestrous cycles and therefore corresponded to the classical description of the problem animals (Roberts, 1971 and Arthur, 1975). The average inter-oestrual period for the 26 animals was 27 days within a range of 14 to 33 days. Majority (16 out of 26) returned to oestrus at longer intervals than 18 to 25 days indicating slightly prolonged periodicity for return to service. Prolonged inter-oestrual period was shown to be suggestive of early embryonic death which constituted the important cause for repeat breeding (Asdell, 1958; Casida, 1961; Bishop, 1964 and Laing, 1970). However, the possibility of early embryonic mortality cannot be ruled out even in animals which returned to oestrum at normal intervals since the embryonic loss has been found to occur well before 10 days after

fertilization (Ayalon et al. 1968; Boyd et al. 1969; Ayalon, 1972 and Ayalon, 1978).

The clinical findings and reproductive history of the experimental animals are furnished in Table 1. Out of the 26 repeat breeders studied, 38.35 per cent had post-partum complications (7 retained placenta and 3 dystokia) in the last calving. Morrow et al. (1969 a&b) showed that post-partum complications like retained placenta, dystokia and milk fever slowed down the process of uterine involution and ovarian activity and animals affected with such conditions required more number of services for conception in subsequent breeding. Namboothiripad and Raja (1972) concluded that chances of repeat breeding increased after gestational accidents and post-partum complications. The present finding is in agreement to these observations and tends to suggest that previous breeding troubles are likely to predispose to repeat breeding. The observations of Arthur (1975) that gestational accidents and post-partum complications delay uterine involution, promoting the chances of genital infection, explains this.

The oestrual discharge of cattle has been described as clear and stringy secretion. Detection of minute flakes or granules of pus or cloudiness in oestrual mucus indicated endometritis of first degree (Dawson, 1960 & 1963; Laing, 1970; Roberts, 1971 and Arthur, 1975). In two of the animals

presently studied there was cloudiness of the oestrual discharge while the rest showed no change in colour. The oestrual discharge was thick in consistency in 16 out of 26 repeaters (61.54%) and only three animals conceived (18.75%) in this group. It is therefore inferred that oestrual mucus with cloudiness and thick consistency indicated increased chances for repeat breeding. Cseh (1963) reported that mucus of sticky and serous consistency showed reduced probability of conception (less than 40%). Studies of Pattabiraman et al. (1967a) showed that mucus of thick and thin consistency maintained sperm motility for lesser time than mucus of medium viscosity. Namboothiripad (1971) also observed that increased thickness of oestrual mucus tends to show reduced chances of conception.

Biopsy

The biopsy instrument used in this experiment was a modification of one designed by Minocha et al. (1964). This instrument consisted of three telescoping tubes designed by Folmer-Nielsen (Kampelmacher, 1954) combined with the speculum principle of Lindley and Hatfield (1952). However, in this modified instrument the diameters of all the tubes were greatly reduced to avoid trauma. The small size of the outer (speculum) tube and its tapered tip permitted ready penetration of the vagina. The tube B, made of stainless steel,

could be readily passed beyond the second or even third cervical ring in the experimental animals. The inner stainless steel tube C could also be worked through the cervix of cows easily during oestrum. The collet on the handle end of the tube A was found to be of considerable advantage in holding the telescoping tubes in position while collecting the sample. The size of the endometrial piece obtained in biopsies varied from 3 to 4 Cu mm, with an approximate thickness of 1 to 1.5 mm. The tissue samples obtained revealed all structural details of endometrium on microscopic studies. The lesions in biopsy samples were considered to represent picture of the whole endometrium as expressed by Hellway (1971).

In an experimental study in 40 cows, Miller (1952) reported that no injuries and haemorrhages or other post-biopsy complications were encountered. Brus (1952) slaughtered and investigated 35 cows after uterine biopsies and observed that the biopsy operation caused only insignificant wounds in the reproductive tract. In the animals, slaughtered after one hour of biopsy, blood appeared in the uterine lumen. It was mostly resorbed in cows slaughtered 24 hours after biopsy. No cellular inflammatory reaction was observed at the wounded spot of endometrium when animals were slaughtered at 36 hours after the operation. Minocha *et al.* (1964) reported that the biopsy could be done repeatedly even

at two to three days interval without introducing extraneous bacteria or producing trauma. Few cases of fibrosis of cervix was observed in heifers on repeated biopsy at luteal stages. In the present study, the uterine biopsies were taken from all the animals during oestrum and only in two cases the oestrual discharge was blood tinged on the following day. In one case there was bleeding which amounted to about 5 ml of blood. In two animals slaughtered 24 hours after biopsy the wound in the endometrium could not be detected. These observations are in agreement to the earlier reports and denote that uterine biopsy could be used as a handy tool for investigations on problem breeder cows without causing in itself any adverse reactions. The insignificant haemorrhage and absence of trauma suggested safety of the modified instrument for routine use.

The processing technique presently employed were at variance to routine histological techniques (Humason, 1972) and from the process earlier described for uterine biopsy (Brus, 1952; Minocha et al. 1964 and Hallway, 1971). The main modifications employed were with regard to the fixation time and the duration of treatments for dehydration and clearing. Treatment for 10 minutes in each of the ascending series of alcohol and short treatment of 2 to 3 minutes in xylene for clearing proved to give best results. Present

study also revealed that Bouin's fluid is a satisfactory fixative for bovine endometrial biopsy specimens. These observations merit further trials.

In the present study, cent per cent conception rate was obtained in the control animals when bred after 8 to 12 hours of biopsy operation. In the experimental animals nine out of 26 repeat breeders settled at inseminations done in the same heat of biopsy sampling. It is therefore inferred that biopsy operation does not affect the structural and functional competance of uterus to sustain pregnancy. Similar observations were recorded by earlier workers. Brus (1952) reported 18 out of 25 repeat breeding cows conceived as a result of insemination immediately after the biopsy. Skjervan (1956) obtained a conception rate of 57.8 per cent in the first service after taking biopsies in 252 cows. He also observed that some of the animals were pregnant on the side from which biopsy had been taken. Mc Queen (1967) also carried out biopsy operations in bovines without seriously altering the conception rate.

Antibiotics were administered intra-uterine to all experimental and control animals after taking biopsy as a protective measure against possible chances of trauma and infection that may be encountered as a result of biopsy. Moreover, intra-uterine antibiotic treatment after

insemination had been claimed to be of advantage in enhancing conception rate in repeat breeder cows (Khan and Luktuke, 1967; Namboothiripad and Mathai, 1970 and Ramadas et al. 1978).

Lesions

The biopsy findings from repeat breeders were combined with historical and physical examination to evaluate the overall fertility of the cow. Pathological changes in the endometrial biopsies were observed in 53.85 per cent of the repeat breeders under study. Changes in the uterus observed in biopsy were significant and consisted of infiltration with various types of inflammatory cells like lymphocytes, plasma-cells, macrophages, eosinophils and neutrophils, periglandular fibrosis, cystic dilatation of glands, glandular hypertrophy, stromal hyalinization and sclerosis. Similar uterine lesions have been described by earlier workers in repeat breeder cows. Brus (1952) observed that 71 out of 100 repeat breeders showed evidence of endometritis on examination of biopsies. He found the inflammatory cells mostly lying under the lining epithelium or concentrated in and around the blood vessels, or in severe cases they formed foci, mostly around glands and blood vessels. Cupps (1973) noticed abnormal uterine glands, morphologically characterised by enlargement of the lumen, various degrees of degeneration of the glandular epithelium, and a localised modification of stroma accompanied by

infiltration of either eosinophils or lymphocytes or both in 55 per cent of the repeat breeder cows. Rao et al. (1975) in a study on repeaters observed periglandular fibrosis, cystic dilatation of glands, lymphoid aggregates in the endometrium alone or in combination indicating low grade infection. These lesions corresponded to the inflammatory lesions described in varying degrees of endometritis (Dawson, 1960 & 1963; Bhandari, 1973; Bhosreker, 1973; Seitardis and Tsangaris, 1973; Zafracas, 1974 and Dawson, 1977).

Cupps (1973) reported that the presence of eosinophils and lymphocytes or in combination in the endometrium was closely associated with modified glands. Eosinophilic infiltration has been reported under several conditions. Murphy (1924) found few number of eosinophils in the endometrium of cows during the entire oestrous cycles with greatest concentration about the fifth day of the cycle. However, Weber et al. (1948) did not encounter uterine eosinophilia in heifers during the oestrous cycle nor during pregnancy. According to Call et al. (1968) uterine eosinophilia occurred following death of the early embryo. All these indicated that high concentration of eosinophils was associated with abnormal conditions of the uterus. Eosinophils probably played an important role in the destruction

of secretions of the occluded glands that were reabsorbed into the endometrium. Lymphocytes also appear to be active in modification of the morphology and activity of the occluded glands. Cupps (1973) opined that functional uterine changes caused by eosinophils and lymphocytes probably were responsible for the changes in the uterine environment which interfered with successful fertilization and implantation and thus accounted for the delay or failure of fertilization and successful gestation in affected animals. In the present study out of 26 repeat breeders lymphocytic infiltration of varying degree was observed in 18 cases and of this, only four animals conceived (22.22%). Muursepp (1972) concluded that a significant correlation between conception and degree of lymphocytic infiltration of the endometrium existed. In his study, 69 cows with weak diffuse lymphocytic infiltration, 22 with moderate or marked diffuse infiltration and 22 with focal infiltration showed conception rate of 69.6, 36.4 and 27.3 per cent respectively.

Plasmacells are seldom observed in normal endometrium. If plasmacells are present in greater numbers or with moderate ease in uterine sections it can be diagnosed as chronic endometritis (Silverberg, 1977). Plasmacytes are the major producers of humoral antibody. Their presence is interpreted to indicate continuing presence of antigen which is presumed to be microbial in nature (Kenney, 1978). Plasmacell

infiltration in the presently studied animals, therefore, indicated chronic endometritis probably infectious origin.

In the present study, out of 26 repeaters, three showed cystic distention of glands. Out of this, two failed to conceive indicating that cystic distension interfered with the ability of the endometrium to sustain the embryo resulting in early embryonic mortality (Moss et al. 1956). Cystic distention of endometrial glands appeared to arise by more than one machanism (Kenney, 1978). He explained that it could be due to the strangling effect produced by periglandular fibrosis or due to lack of tone and peristaltic action of myometrium resulting in accumulation of secretions, or due to epithelial hypertrophy.

Periglandular fibrosis was observed in three cases out of 26 animals examined during the course of this investigation. All the three animals failed to conceive even after biopsy and treatment. It could be inferred that periglandular fibrosis interfered with conception. It appeared to compromise the functional activity of the involved gland. Extent and severity of fibrosis directly affected the ability of the uterus to conceive. When fibrosis was severe, it reduced the ability of the uterus to conceive (Moss et al. 1956 and Cupps, 1973). Stromal cells of the lamina propria normally do not form collagen. However, the ability to

deposit collagen in response to chronic inflammation as well as other stimuli has been shown (Kenney, 1978).

The lesions observed by examination of endometrial biopsy of the repeat breeder cows presently studied denoted inflammatory reaction of varying degree. Some of the lesions were suggestive of infection of uterus with resultant endometritis. It has been possible to locate microbial organisms in the tissue section in one case. The present findings therefore indicate that endometritis of infectious nature constituted the most important lesions in repeat breeder cows. However, endometritis was not apparently detectable on routine clinical examination. The present observations is in confirmity with the opinion of many earlier workers (Brus, 1952; Miller, 1952; Moss et al. 1956; Hartigen et al. 1972; Cupps, 1973; Rao et al. 1975; Namboothiripad, 1976 and Namboothiripad and Raja, 1976).

Interpretation of histologic findings

Based on histologic findings, the animals were classified into three groups (Table 5). In group I, animals with normal or nearly normal endometrium were included. Presence of few mononuclear cells were considered normal (Murphy, 1924 and Weber et al. 1948) and were included in group I. Twelve out of 26 repeat breeders (46.15%) came under this group. The

percentage of conception in this group (58.33%) compared well with herd averages for any well managed herd. Animals which showed more extensive inflammatory lesions were classified into group II, which showed a conception rate of only 25 per cent. It appeared that such inflammatory change and slight fibrotic changes were sufficiently severe to interfere with the ability of the endometrium to support a foetus to term. Six out of 26 repeat breeders (23.08%) showed more severe endometrial lesions and were included in group III. It was observed that no animals in this group conceived. The present study revealed that majority (53.85%) of repeat breeders were affected with endometritis which could be detected by uterine biopsy. These observations are in general agreement to the findings of Brus (1952).

It could be summarised that by obtaining the uterine biopsies, degrees of pathological changes could be assessed to help in predicting the chances of conception. Similarly treatments could be suggested on this basis. The present study shows that uterine biopsy is a valuable aid in diagnosis and treatment of repeat breeder problem. Though majority of the presently studied animals showed uterine pathology to explain the failure of conception, no reasons for failure of breeding could be detected in 19.23 per cent of animals by the biopsy technique. It could be summased that these animals failed to settle on account of reasons which

were not reflected in uterine biopsy.

Observations on slaughtered animals

In order to evaluate the merits of the biopsy findings, seven out of 17 repeaters which failed to conceive after the operation and treatment were slaughtered and their genitalia were subjected to detailed objective investigations. It was intended to test the reliability of the biopsy examinations and to correlate other lesions, if any, which were not revealed by biopsy. It could be observed from Table 6 that the ovaries in all cases appeared normal with signs of oestrual activity. Corpus luteum of varying sizes were observed in one of the ovaries in all cases. In one case, there was presence of thin fibrous strands between the ovary and the bursa. It could be concluded that the repeat breeder cows examined during this study showed normal cyclic activity of ovaries with no significant lesions to impair reproductive efficiency.

Two out of seven cows slaughtered showed unilateral thickening and blockage of the salpinx (Table 6). Histopathological examination of sections revealed vacuolation and degeneration of epithelial cells in one case and lymphocytic cells within the lumen in the other. Cembrowicz (1950) observed fairly high incidence of salpingitis in endometritis

cases and expounded the theory of ascending infection. Moberg (1954) stated that majority of the salpingeal lesions were due to descending infection from the areas of haemorrhage in the ovary occurring at ovulation or at enucleation of corpus luteum or rupture of cystic follicles. The blockage of salpinx may result from chronic inflammation too (Nair and Raja, 1974). Namboothiripad et al. (1978) noticed salpingitis and blockage of fallopian tube in association with endometritis in repeat breeder buffaloes. Since the co-existence of endometritis and mild salpingitis with blockage of tube were the lesions in the present cases it is suggestive of ascending infection from uterus and resultant inflammation.

In the present study, the uterus of all the animals slaughtered showed varying lesions on histological examination. In two cases, congested areas and mucopurulent coating on the surface was apparent on gross examination. In one case, the lumen contained yellowish pus with decaying embryo towards the middle of the left horn. This animal had a history of breeding 15 days before. Other reproductive organs did not reveal any gross changes though lesions were detectable on microscopic examination. In one case, oedema and swelling with slight mucopurulent coating was seen in the cervix as well.

Sections of the uterus revealed inflammatory changes

of varying degree in all cases. Mononuclear cell infiltration, predominently periglandular and perivascular constituted common lesion. Periglandular fibrosis, focal cystic dilatation with desquamation of lining cells of glands and stromal hyalinization were also observed in few cases. It could be seen from Tables 3 and 7 that autopsy findings correlated with the biopsy lesions in six out of seven cases. In one case (Cow No. 429), however, the biopsy did not reveal significant inflammatory changes while on autopsy marked lesions were found. It could be inferred that the presently employed biopsy technique adequately reflected the uterine pathology.

The pathological changes presently observed were similar to the one described by Moss et al. (1956), Cupps (1973), Bhandari (1973), Rao et al. (1975) and Namboothiripad (1976) in repeat breeder cows and buffaloes. The tissue changes were severe enough to endanger the growing zygote. Endometritis due to uterine infection of the magnitude as in present cases has been shown to create unfavourable environment for the developing embryo (Lindley, 1954; Succhi et al. 1956; Hinze, 1959; Rowson et al. 1972; Sreenan and Beehan, 1974; Namboothiripad, 1976 and Namboothiripad and Raja, 1976). Boyd (1965) while reviewing early embryonic mortality in cattle projected several evidences to suggest that endometritis was the common cause for early embryonic loss. The mild cervicitis could be on account of biopsy

(Minocha et al. 1964). In the present study, no pathological changes of vulva and vagina were observed in repeat breeders in striking variation to the views of Rao et al. (1975).

The present study denotes that uterine pathology characterised by endometritis constituted the predominent lesion in repeat breeder cows. Presence of bacterial clumps in the lamina propria, infiltrating cells, and other inflammatory responses suggest the possibility of infection to be the cause of endometritis. Endometrial biopsy proved to be useful tool to diagnose this condition which could otherwise go unnoticed. This is in keeping with the earlier reports of Brus (1952), Miller (1952) and Minocha et al. (1964).

Endometrial biopsy has been proved to be of value in experimental and diagnostic purposes in mares as well (Kundsen, 1964; Brandt and Manning, 1969; Witherspoon and Goldston, 1972; Rickets, 1975a&b; Borgman and Kenney, 1976; Nitschelm and Horst, 1976; Gordon and Sartin, 1978; Konney, 1978 and Platt and Dunbar, 1978). From the studies undertaken on endometrial biopsies in cattle in this project, it can be concluded that as in the case of mares, the endometrial biopsy technique could be profitably employed in evaluating the endometrial lesions, which are particularly of a subclinical nature.

SUMMARY

SUMMARY

The main objective of the present investigation was to study the uterine pathology in repeat breeding cows employing uterine biopsy as a diagnostic tool for tackling the problem. A biopsy instrument with minor modifications of the one designed by Minocha et al. (1964) was used for this purpose. The studies were carried out on 26 repeat breeding cross-bred cows from the herd of the University Livestock Farm, Mannuthy which were under regular sexual health control programme. In addition, four cows which settled at first insemination were taken as control animals for comparison. Biopsy of the uterus was taken from all the repeat breeding and control animals during the early part of heat. Every cow was inseminated 8 to 12 hours after biopsy with good quality semen and was treated with one gram streptomycin and 8 lakh units penicillin in 30 ml sterile distilled water 12 to 24 hours after insemination. The biopsy specimens were processed for histological studies by developing a suitable procedure after trial and error. Seven out of 17 animals which failed to conceive even after biopsy and treatment were slaughtered and their genitalia were subjected to detailed studies.

The tissue samples obtained by the biopsy instrument revealed all structural details of endometrium and no

post-biopsy complications were encountered except for insignificant bleeding. In the experimental animals, nine out of 26 repeat breeders and all the four control animals conceived at artificial insemination in the same heat of biopsy explaining the fact that biopsy operation did not affect the functional status of uterus to sustain pregnancy.

Pathological changes in the endometrial biopsies were observed in 14 out of 26 (53.85%) of the repeat breeding cows. Changes in the uterus observed in biopsy were significant and consisted of infiltration with various types of inflammatory cells like lymphocytes, plasmacells, macrophages, eosinophils and neutrophils, periglandular fibrosis, cystic dilatation of glands, glandular hypertrophy, stromal hyalinisation and sclerosis. It was possible to detect signs of inflammation of infective nature. Endometritis, therefore, constituted the most important lesion in the repeat breeders presently studied. The lesions were severe enough to compromise the functional competance of uterine glands in particular and the endometrium in general to sustain pregnancy.

Based on histological findings the animals were classified into three groups - group I showing normal or nearly normal endometrium, group II with more extensive inflammatory changes and group III with severe inflammatory and fibrotic changes. The biopsy picture in group I was

similar to that in control animals which conceived at first service. Group I and II had conception rates of 58.33 per cent and 25 per cent respectively. In group II, none conceived. Results of insemination in control animals and groups I and II revealed that operation to collect biopsy specimen was a safe technique with no ill effects on chances of conception. The lesions in the slaughtered animals corroborated with biopsy findings in general.

In the present study uterine lesions were interpreted on the basis of breeding record, clinical findings, biopsy lesions and autopsy findings. The following inferences were drawn:-

1) The repeat breeding cows presently studied had normal oestrus period with majority (16 out of 26) showing prolonged periodicity of return to service.

2) Biopsy operation was found to be a reliable tool to study the physio-anatomical competance of the uterus in cows, with no significant side effects on the reproductive efficiency of the subjects.

3) Majority (53.85%) had endometritis and this was attributed as the reason for failure of conception. It was concluded that 19.23 per cent animals which showed no uterine lesions were repeaters due to reasons undetected.

4) It could be collaterly inferred that post-insemination antibiotic therapy was curative in treatment of repeaters since infectious endometritis was the lesion in majority of cases.

5) Uterine lesions characterised by severe cellular infiltration and glandular changes, represented probably neglected cases. These were not amenable to treatment. It amounted to suggest the need for early detection and treatment of uterine inflammatory conditions. This observation stressed the need for early detection and treatment of uterine inflammatory conditions.

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STUDIES ON UTERINE PATHOLOGY IN REPEAT BREEDING CATTLE

BY

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ABSTRACT OF A THESIS

Submitted in partial fulfilment
of the requirement for the degree

MASTER OF VETERINARY SCIENCE

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1979

ABSTRACT

An investigation was undertaken to study the uterine pathology in repeat breeding cows employing uterine biopsy as a diagnostic technique. The studies were carried out in 26 repeat breeding cross bred cows from the herd of University Livestock Farm, Mannuthy using a biopsy instrument which was a modification of one designed by Minocha et al. (1964). Four cows which settled at first insemination served as control animals for comparison. From all the animals biopsy was taken during the early part of heat and was inseminated 8 to 12 hours after biopsy followed by intrauterine antibiotic therapy after 12 to 24 hours. Seven out of 17 animals which failed to conceive even after biopsy and treatment were slaughtered and their genitalia were subjected to detailed studies.

Nine out of 26 repeat breeders and all the four control animals conceived at inseminations in the same heat of biopsy explaining the fact that biopsy operation did not affect the functional status of uterus. Pathological changes in the endometrium were observed in 14 out of 26 (53.85%) repeat breeding cows. Changes in the uterus observed in the biopsy were significant and consisted of infiltration with various types of inflammatory cells, periglandular fibrosis,

cystic dilatation of glands, glandular hypertrophy, stromal hyalinization and sclerosis. It was seen that endometritis constituted the most important lesion.

The experimental animals were classified into three groups based on histological findings; group I showing normal or nearly normal endometrium, group II with more extensive inflammatory changes and group III with severe inflammatory and fibrotic changes. Group I and II had a conception rate of 58.33 and 25 per cent respectively. In group III, none conceived. Results of insemination revealed that uterine lesions characterized by severe cellular infiltration and glandular changes affected the chances of conception adversely. The lesions in the slaughtered animals correlated with biopsy findings in general.