THERAPEUTIC MANAGEMENT AND CHANGES IN THE ENDOMETRIUM OF REPEAT BREEDING COWS*

J.A. PATEL[1], A.J. DHAMI[2], F.S. KAVANI[3], M.T. PANCHAL[4] and D.J. GHODASARA[5]

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary Science and Animal Husbandry,
Anand Agricultural University, Anand-388 001, India

ABSTRACT

The overall incidence of repeat breeding observed among 98 breedable Holestein Friesian (HF) animals of a farm was 40.82 % (n=40). These 40 repeat breeding cows were divided at random in different groups with a view to evaluate the relative efficacies of different hormonal therapies at breeding towards improving their reproductive efficiency over the control. The conception rate following i/m treatment at the time of insemination with GnRH (Receptal, Buserelin acetate, 0.02 mg), LH (Chorulon, 1500 IU) and Progesterone (Proluton depot, 500 mg) was 66.66, 83.33 and 50.00 %, respectively, with an overall mean of 66.66 % (12/18). This was significantly higher (P<0.01) than that (33.33%, 2/6) obtained in control untreated repeat breeding cows. The overall conception rate in the treatment cycle itself was significantly (P<0.01) higher than in untreated control group (55.55 vs 16.66%). Further, out of 10 chronic repeat breeding cows treated with $PGF_{2\alpha}$ (Vetmate, 2 ml) at mid-cycle, 9 (90%) cows came in estrus within 80 hrs of treatment and 33.33 % (n=3) conceived at first estrus following GnRH treatment (0.01 mg i/m) at second insemination 12 hrs apart. Out of 18 endometrial biopsy samples collected from right and left horns and body of the uterus of 6 culled repeat breeding cows, chronic endometritis was detected in 12 samples (4 animals) and sub-acute endometritis in 6 samples (2 animals). No significant difference could be observed in histopathological changes pertaining to endometrial biopsies from left and right uterine horns and uterine body. Although almost identical changes were seen at all the 3 sites of the uterus, however, microscopic lesions between animals differed. The endometrial biopsy proved a good guide to decide the prognosis of chronic repeat breeding animals and GnRH or LH can be recommended along with AI to improve fertility in infertile cows.

Key words: Endometrial biopsy, Hormonal therapy, Repeat breeding cows.

INTRODUCTION

Repeat breeding syndrome is one of the frustrating gynaecological maladies of the dairy animals. It is one of the main reasons of infertility and has long been a problem world wide to dairy farms (Ayalon, 1984). This syndrome leads to loss of time and economy due to the decreased conception rates in dairy cattle. The incidence of repeat breeding ranges from 4.26 to 42.70 % in different managemental herds. Many factors, viz. season, breed, parity, periparturient diseases and milk yield have been reported to influence the occurrence of repeat breeding in dairy cattle. Hormonal and non-hormonal therapies are used by practicing veterinarians for this malady.

GnRH and/or LH is known luteotropic hormone, which strengthens the luteal activity in problem breeders. As progesterone is responsible for maintaining a quiescent favourable environment in the

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[1]Associate Professor, [2]Professor & Head, [3]Retired Professor & Head, [4]Professor, [5]Associate Professor, Department of Veterinary Pathology.
corresponding author: ajdhami@aau.in ; dhami_1659@yahoo.com

uterus for embryo development, it is logical to consider that supplementation with this hormone may improve conception rate in repeat breeding cows. $PGF_{2\alpha}$ is highly effective in regulating estrous cycle by inducing complete luteolysis. Hence, it may be of interest to examine the feasibility of injecting GnRH or hCG at the time of AI following $PGF_{2\alpha}$ induced estrus for improving fertility in such repeat breeder cows. Moreover, all uterine abnormalities cannot be detected grossly by routine clinical examination. Hence, microscopic study of the infertile reproductive tracts is necessary to detect inflammatory or degenerative reactions, which may change the uterine environment. The present study was aimed to touch these objectives in repeat breeding exotic cattle under tropical climate.

MATERIALS AND METHODS

The present investigation was carried out in the university herd of Holstein Friesian cows of IDC Unit, Anand. In all, 98 breedable animals were screened thoroughly for their genital health and reproductive status through past records and gynaeco-clinical examinations per rectum. The repeat breeding cows (n=40) which had no palpable or visible genital abnormalities were further subdivided in smaller groups and studied for their response to hormone therapies (n=34) and changes in endometrial biopsies (n=6). First 24 repeat breeding cows were randomly divided into 4 treatment groups, each of 6 animals, as follows.

Group I: Cows treated with single i/m injection of 0.02 mg buserelin acetate (GnRH analogue; 5 ml Receptal, Intervet India Ltd.) immediately after insemination.

Group II: Cows treated with single i/m injection of 1500 IU hCG (Chorulon, Intervet India Ltd.) immediately after insemination.

Group III: Cows treated with 500 mg of progesterone (Proluton depot, German Remedies India Ltd) i/m on 6th day of insemination.

Group IV: Cow inseminated at estrus without any treatment served as untreated control.

Moreover, in animals of all these groups, injection Gentamicin 15 ml was given intrauterine 10-12 hrs after insemination to check genital infection, if any, and all were followed for return to estrus or conception. Pregnancy was confirmed per rectum 60 days post-insemination and findings were compared statistically between groups.

Prostaglandin Plus GnRH treatment in Repeat Breeder Cows

Ten repeat breeding HF cows, which did not conceive by previous GnRH or hCG therapy up to 3rd service, were injected Vetmate, 2 ml i/m, (Cloprostenol, a synthetic analogue of $PGF_{2\alpha}$, Tetragon Chemie Pvt. Ltd.) on 10th day of estrous cycle for induction of estrus and were inseminated twice at 12 hourly interval with good quality frozen thawed semen followed by Receptal (GnRH analogue) 2.5 ml i/m immediately after second AI. The interval for onset of estrus, its duration, intensity (intense, medium, weak) and fertility (per rectum, 60 days post-AI) were evaluated to study the effect of this regime.

Histopathological Examination of Endometrial Biopsy

Six culled chronic repeat breeding cows (repeating beyond one and a half year postpartum) were selected for this purpose and endometrial biopsies were taken aseptically from the left and right uterine horns as well as from the body of uterus using Nelson's biopsy punch for detection of histopathological changes. The stage of estrous cycle was confirmed per rectum by palpation of follicle or CL on the ovary before taking biopsy. The biopsy samples (4-5 mm) were fixed in 10% formalin. The cryosections were cut by microtome machine and were stained with H & E stains to visualize the microscopic details.

RESULTS AND DISCUSSION

A total of 98 breedable exotic animals of HF farm screened per rectum revealed the incidence of repeat breeding condition to be higher in cows (43.23 %) than the heifers (33.33 %) with the overall mean of 40.82 per cent. These results corroborated with the findings

of Bharkad and Markandeya (2003). However, Bhosrekar (1973) reported higher incidence of repeat breeding in heifers than in cows. Moreover, the incidence of repeat breeding observed in the present study was higher than that (8.39-28.38%) reported by Chetty and Rao (1987) and Selvaraj *et al.* (2003).

Therapeutic Management of Repeat Breeding Cows

The pooled conception rates of 3 cycles obtained in repeat breeding cows following single i/m injection of GnRH, hCG and Progesterone (Proluton) after insemination at first cycle were 66.66 (4/6), 83.33 (5/6) and 50.00 (3/6) per cent, respectively, with an overall conception rate of 66.66 (12/18) per cent. This was significantly higher ($P<0.01$) than 33.33 % (2/6) conception rate obtained in untreated control repeat breeding cows. In GnRH group, 50.00 (3/6) per cent cows conceived in the treatment cycle itself and 16.66 (1/6) per cent in II post-treatment cycle. Whereas in hCG group, 66.66 (4/6) per cent cows conceived in treatment cycle and 16.66 (1/6) per cent conceived in I post-treatment cycle. In progesterone group, only 50.00 (3/6) per cent animals conceived in treatment cycle, but none in I or II post-treatment cycle. The overall conception rate in the treatment cycle itself was significantly ($P < 0.01$) higher than that in untreated control group (55.55 vs 16.66 per cent).

The higher conception rate (66.66 %) obtained with GnRH treatment compared to control (33.33 %) corroborated well with many of the previous reports (Shelar *et al.*, 2002). Moreover, Pathak *et al.* (1986) obtained significantly higher conception rate in repeat breeding cows following GnRH treatment at the time of AI than in untreated control group (47.0 vs 37.7 %) and similar were the observations of Rayos (1995) but with relatively higher conception rates in both treated and untreated groups (70 vs 50%) of repeat breeding cows. GnRH-agonist has a luteotropic effect that delays regression of corpus luteum, maintains high progesterone concentration and improves conception rates by 8-10% in repeat breeding cattle (Gonzalez *et al.*, 1999). The results of the present study denote that GnRH can be used immediately after AI to improve fertility in infertile female bovines. The dose of GnRH presently used (0.02 mg) perhaps appeared appropriate as Ata and Tekin (2001) also obtained significantly higher conception rate (60 vs 30 %) in repeat breeding cows using 0.02 mg than the 0.01 mg dose of GnRH.

Significantly higher conception rate obtained with hCG treatment in repeat breeding cows as compared to control group (83.33 vs 33.33 %) coincided well with the report of Ingawale *et al.* (2002). It was also nearer to 70.0 vs 37.5 % conception rate obtained with similar treatment protocol by Srivastava and Ahlawat (1998). The improvement in conception rate obtained in repeat breeding cows treated with hCG might be due to its regulatory effect on time of ovulation as well as its luteotropic effect (Srivastava and Ahlawat, 1998). The hCG has an established beneficial effect on fertilization, corpus luteum development, implantation of embryo and its survival and hence successfully used by most practitioners in problem breeders.

The 50 per cent conception rate recorded in progesterone treated repeat breeding cows as compared to 33.33 % in untreated control group was in agreement with the reports of Das *et al.* (2002), but was lower than that (66.7 vs 50.0 %) reported by Sharma *et al.* (2003) in repeat breeding buffaloes. Awasthi *et al.* (2002) also reported significant improvement in conception rates (66 vs 48 % and 70 vs 20 %, respectively) of repeat breeding cows following progesterone supplementation on day 4 to 10 post-breeding. Embryonic loss before 15 days of gestation is usually preceded by and may be caused by luteal regression. Hormonal treatment to increase plasma progesterone level may improve pregnancy rates, particularly in repeat breeding cattle (Kastelic, 1994).

Effect of Prostaglandin plus GnRH in Chronic Repeat Breeding Cows

Out of 10 chronic repeat breeding cows, which failed to conceive with previous GnRH or hCG therapies, when treated with $PGF_{2\alpha}$ at mid-cycle, 9 (90 %) came in estrus within 80 hrs of treatment with different intensity and duration. They were also injected with 2.5 ml Receptal i/m at the time of second insemination (2 AI, 12 hrs apart), which resulted in conception in 3 (33.33 %) cows. In this study, the dose of $PGF_{2\alpha}$ used

and luteal stage at which the cows were treated may be optimum to cause better luteolysis to set LH surge for inducing estrus. The frequency of intense heat in repeat breeding cows was more (66.66%) as compared to medium (22.22%) and weak (11.11%) heat. Goley and Kadu (1995) also reported similar observations.

The first estrus/service conception rate obtained in this study (55.55 %) is in accordance with the findings of Goley and Kadu (1995), but was lower than 62.5 % reported by Selvaraj *et al.*,(2004) using same treatment protocol in repeat breeding cows. The result achieved in our study was challenging as the repeat breeding cows used in the trial were of long standing and they were likely to be culled, if not conceived. Further, the double inseminations performed at induced estrus with quality semen might have improved the pregnancy rate of chronic repeat breeding cows under study, which did not respond to previous hormonal therapy.

Histopathological Changes in the Endometrial Biopsies of Chronic Repeat Breeding Cows

Histopathological examinations of endometrial biopsies of left and right uterine horns as well as the body of the uterus of 6 chronic repeat breeding cows (1 in follicular phase and 5 in luteal phase) revealed almost similar changes in all the three sites of uterus. No significant difference could be observed in histopathological changes in endometrial biopsies collected from three different sites of the uterus of the same animal.,However, the lesions varied between animals. Out of 18 biopsy samples collected from 6 animals, chronic endometritis was detected in 12 samples (4 animals) and sub-acute endometritis in 6 samples (2 animals).

Microscopic lesions of sub-acute endometritis were characterized by degeneration of glandular epithelium with inspissated secretions and cellular remnants in lumen and mononuclear cell infiltration (Fig. 1), aggregation of lymphocytes in the endometrial stroma (Fig. 2) and mild to severe degeneration of stromal blood vessels. The common histological pictures of chronic endometritis were extensive stromal, perivascular and periglandular fibrosis (Fig. 3), and cystic dilatation of endometrial glands (Fig. 4). These histopathological changes were similar to those reported by Gonzalez *et al.*,(1985) and Prajapati (2003). Severe periglandular fibrosis in chronic endometritis play an important role in the retardation of uterine milk secretion and alteration in protein synthesis, consequently leading to early embryonic death, infertility and repeat breeding (Gonzalez *et al.*, 1985). Biopsy studies of endometrium proved a good diagnostic tool for the diagnosis of endometritis and predicting the prognosis of chronic repeat breeding animals.

It was concluded that a significant number of repeat breeding cows could be settled by systematic investigation and proper therapeutic measures. Both hCG (83.33 %) and GnRH (66.66 %) improved conception rate over untreated control (33.33 %) suggesting their beneficial role, if used at the time of AI, in fertility enhancement of repeat breeders. Histopathological evaluation of endometrial biopsies proved valuable in detecting the severity of lesions and in predicting the prognosis of chronic repeat breeding animals. However, further studies on large number of repeat breeding animals in each group, particularly on therapeutic aspects, are warranted to draw valid conclusions.

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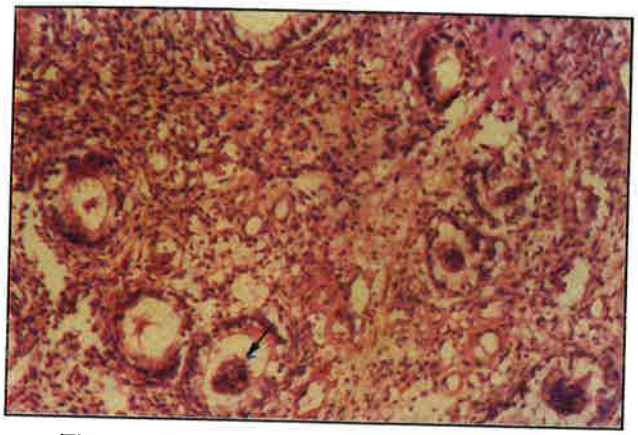

Fig. 1. Sub-acute endometritis showing degeneration of glandular epithelium with inspissated secretion and cellular remnants in lumen (arrow) and mononuclear cell infiltration (H & E X 950)

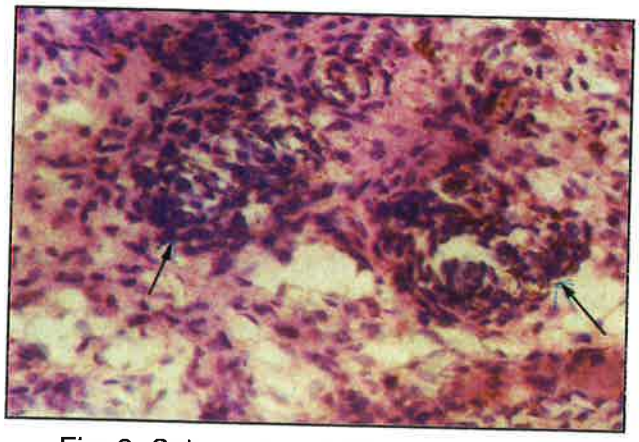

Fig. 2. Sub-acute endometritis showing aggregation of lymphocytes in the endometrial stroma (H & E stain X 1900)

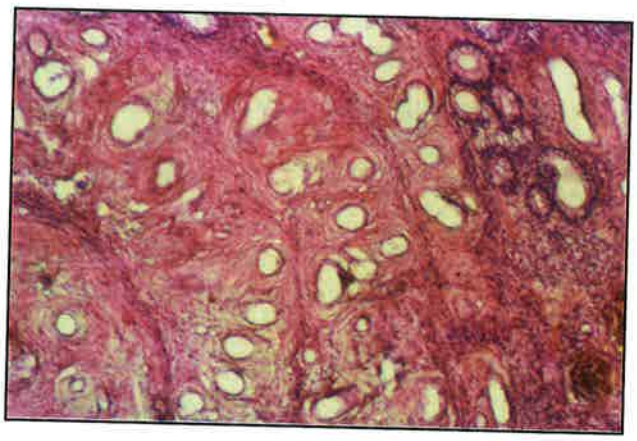

Fig. 3. Chronic endometritis showing extensive perivascular and periglandular fibrosis (H & E X 950)

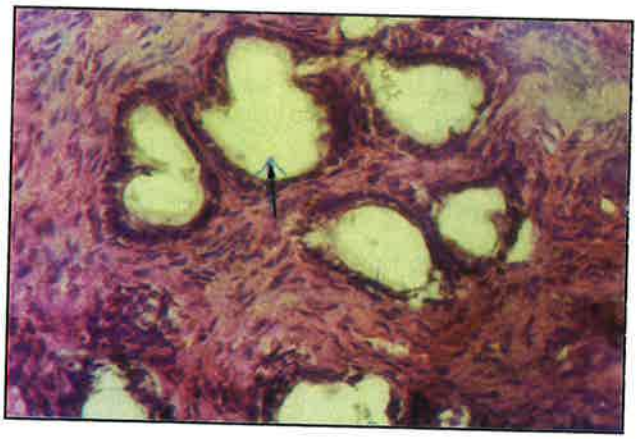

Fig. 4. Chronic endometritis showing cystic dilatation endometrial gland (H & E X 950)