

## AUGMENTATION OF FERTILITY IN POSTPARTUM ANESTRUS COWS

A.K. TRIPATHY, D.N. MOHANTY\*, S. DAS AND P.C. MISHRA

Department of Animal Reproduction, Gynaecology and Obstetrics,  
College of Veterinary Science and A.H., O.U.A.T, Bhubaneswar-753 003

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### ABSTRACT

The main objective of the study was to augment fertility in postpartum anestrus cows by application of Progesterone therapy alone or in combination with Estrogen through parental (i/m) route. Thirty postpartum anestrus cows allotted to three equal groups were administered intramuscularly progesterone 500 mg (P- Depot) and estradiol valerate 10 mg (Progynon- depot) on day "0" followed by P-Depot on 7th day (Group I n-10). The Group II (n-10) received P-Depot on day "0" followed by Progesterone and estrogen therapy on day 7. But Group III (n-10) cows were given only progesterone on corresponding days. Serum P4 concentration was significantly higher ( $P < 0.01$ ) in cows on 7th day post-treatment ( $5.34 \pm 1.74$  ng/ml) compared to pre-treatment value ( $1.98 \pm 1.09$  ng/ml). The estrus response was 100% in Group II followed by 90% in Group I cows. The duration of estrus (hrs) was significantly longer in Group II ( $28.90 \pm 0.82$ ) compared to Group III ( $21.80 \pm 2.00$ ) but not against Group I ( $25.77 \pm 1.78$ ). The estrual characteristics (1-4) revealed higher rating values in Group II cows ( $2.70 \pm 0.32$ ) followed by Group I ( $2.67 \pm 0.37$ ) and Group-III ( $1.80 \pm 0.83$ ). The overall conception rates in group I, II and III cows 77.78(7), 80.00(8) and 60.00(3) per cent respectively, with significant difference between Group II and Group III animals. It is concluded that progesterone and estradiol combination therapy due to their synergistic effect can give higher overall conception rate in postpartum anestrus cows.

**Key words:** Post partum Anestrus, Estradiol valerate, P-Depot, Conception rate

### INTRODUCTION

Generally the increased partitioning of energy to milk production results in postpartum anestrus by delaying resumption of follicular activity. However, factors such as limited energy intake, lower body reserves and post partum diseases also delay the return to cyclicity. In suckling cows the release of GnRH is diminished due to suppressive action of either prolactin or  $\beta$ -endorphins on hypothalamus. In many cases though there is initiation of cyclic activities but exhibition of pronounced estrus is not noticed. It is desirable that cows should conceive within 90 days of post partum so as to ensure optimum production. The sensitivity of the hypothalamus to the negative feed back effect of progesterone therapy results in increased gonadotropic stimulation, the largest ovarian follicle matures and responds to exogenous estradiol or a gonadotropin. Moreover, behavioral centers are activated, enabling expression of overt estrus in a large proportion of animals (Rhodes *et al.*, 2003).

### MATERIALS AND METHODS

Crossbred cows (n-30) with a history of postpartum anestrus for > 90 days and of 1st to 5th parity with normal genitalia and smooth/ functional ovaries were considered for the investigation. These 30 cows were allotted to 3 different groups consisting of 10 cows in each. The Group I animals (true anestrus with smooth and functionless ovaries and quiescent reproductive tract) were administered with two doses of P-Depot comprising of 500 mg progesterone each through i/m route at an interval of 7 days along with estradiol valerate 10 mg (Progynon depot, Zydus- Cadila) i/m on the 1st day. The Group II animals (functional anestrus with persistent CL/ cystic corpora lutea and exhibiting silent/ unobserved estrus) received two doses of P-Depot as above along with estradiol valerate 10 mg i/m on 7th day. Group III animals (either true anestrus or functional anestrus) were injected only two doses of progesterone (P-Depot) 500 mg each i/m at an interval of 7 days. Estrus response was then evaluated and cows in confirmed estrus were inseminated with frozen semen.

The cows were bled by jugular vein puncture and 10-15ml of blood was collected without anticoagulant. Serum harvested by standard procedure was stored in

\*Professor & Head and corresponding author- E mail mohantydn@gmail.com

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separate sterile vials at  $-20^{\circ}\text{C}$  for estimation of progesterone level. The blood samples were collected from experimental animals on day '0' (pretreatment) before estrogen/ progesterone injection and on day 7 before administration of 2nd progesterone/ estrogen injection.

Characteristics of induced estrus were estimated by induction interval; duration of estrus (in hrs) and grading or rating of estrus was done after evaluating the estrus behaviour of individual animal and graded from 4 to 1 as under.

4 : Clear, rosy, vaginal discharge, frequent vocalization, motor movement and excitation.

3 : All the characteristics of estrus with less intensity.

2 : Clear vaginal discharge with no other behavioural symptoms.

1 : None of the external characteristics of estrus is noticed except cervical relaxation and presence of follicle on per rectal examination.

The cervical mucus samples were collected as per the method described by Reddy *et al.*, (1977). Evaluation of colour, fern pattern (Luktuke and Roy, 1967) and measurement of spinnbarkeit (Panigrahi, 1964) were performed from the cervical mucus as per the standard procedure with slight modification of fern pattern, which was graded from 4 to 1 as under.

4 : Extensive prominent branching and sub-branching.

3 : Branching characteristics comparatively less prominent

2 : Major branching present but sub branching less discernable.

1 : Branches and sub-branches qualitatively inferior to others.

Serum progesterone concentration was assayed by direct immuno-enzymatic technique using monobind progesterone ELISA kit in ELISA reader. The monobind progesterone ELISA kit uses a specific anti-progesterone antibody and does not require sample extraction of serum or plasma. The absorbance was read at 450 nm by using a Multilabel Plate Reader (Perkin-Elmer; Germany) of a reference wave length of 620-630 nm. The results were read within 30 minutes of adding the stop solution.

## RESULTS AND DISCUSSION

The average pre-treatment concentration of progesterone (ng/ml) was  $0.78 \pm 0.215$ ,  $1.98 \pm 1.09$  and  $0.219 \pm 0.172$  for Group I, Group II and Group III animals, respectively whereas the corresponding post-treatment progesterone values were  $2.71 \pm 0.40$ ,  $5.34 \pm 1.74$  and  $3.86 \pm 0.936$  for Group I, Group II and Group III cows, respectively. The estrus response consequent to hormonal therapy was 90.00 (9), 100.00 (10) and 50.00 (5) per cent in Group I, Group II and Group III cows respectively. The post-treatment estrus induction interval (hrs) was shortest ( $122.33 \pm 14.06$ ) for Group I ( $122.90 \pm 8.50$ ) for Group II and was longest ( $157.60 \pm 9.70$ ) for Group III animals. The analysis of variance revealed significant difference ( $P < 0.05$ ) with respect to estrus induction interval.

The duration of estrus (hrs) was longer in Group-II ( $28.90 \pm 0.82$ ) as compared to Group-I ( $25.77 \pm 1.78$ ) and Group-III ( $21.80 \pm 2.00$ ) animals. The analysis of variance revealed a highly significant difference ( $P < 0.01$ ) between Group II and Group III but not between Group I and Group II or Group I and Group III. The comparison of estrus grading (1 to 4) showed comparatively higher rating value  $2.70 \pm 0.32$  in cows of Group II. The values for group I and Group III were observed to be  $2.67 \pm 0.37$  and  $1.80 \pm 0.83$ , respectively. However, the analysis of variance did not reveal any significance. Animals of Group II had a fern pattern graded to be  $2.70 \pm 0.55$  which was higher as compared to the animals belonging to Group I ( $2.30 \pm 0.37$ ) and Group III ( $1.80 \pm 0.37$ ). The values did not differ statistically.

The average spinnbarkeit values (cm) of the cervical mucus measured during the estrus of the animals belonging to Group I, Group II and Group III were  $8.00 \pm 0.70$ ,  $8.20 \pm 0.51$  and  $7.00 \pm 0.54$  respectively, which did not reveal any significant difference.

The overall conception rates following estrus induction by various hormonal protocols were 77.78 (7), 80.00 (8) and 60.00 (3) per cent in Group I, Group II and Group III cows, respectively. However, the corresponding first service conception rates were 55.55 (5), 60.00 (6) and 40 (2) per cent in three groups.

The estrus response is in close agreement with the reports of Andurkar and Kadu (1995) and Selvaraju and Rajasundaram (1999). In this study it is evident that progesterone therapy in combination with estradiol has a synergistic effect on the hypothalamic-pituitary-ovarian axis for induction of estrus in postpartum anestrous cows as a result 90 per cent cows in Group I

and 100 per cent in Group II could exhibit estrus. In chi-square analysis, the overall estrus response was significant ( $P < 0.05$ ). The duration of estrus is species dependant and varies slightly from one female to another within the same species (Hafez, 2000). The findings of the experiment corroborates with the observations of Andurkar and Kadu (1995). The cervical mucus, owing to its unique biophysical characteristics shows several rheologic properties such as elasticity, viscosity, thixotropy, tack and fern pattern. The present value of spinnbarkeit is in accordance with Panigrahi (1964) who claimed that a range of 5 to 12 cm favoured conception and maximum spinnbarkeit value was observed at the time of encompassing optimum fertility. This finding also corroborates with the findings of Rao and Rao (1982) and Meheta *et al.*, (1986). The moderate increase in spinnbarkeit value for Group II cows could have augmented fertility. A modified version of fern pattern grading ranging from 4 to 1 according Bane and Rajakoski (1961) has been considered instead of earlier typing like typical, atypical or nil (Fig) Umashanker *et al.*, (1984) observed that the fern pattern was mostly typical with tertiary branching in normal group, while in repeat breeders, it was scattered, small in size and the branching was also thin and fine. The crystallization pattern is due to change in the blood level of ovarian steroid mostly estrogen. Highest crystallization pattern is visible during peak estrogenic phase at the time of standing estrus, estrogen, being an epitheliotropic hormone, increases blood flow and vascularity, causing more secretion from the uterine mucosa. Various electrolytes mostly sodium, potassium, water and lipoprotein are secreted. The discrepancy in crystallization pattern may be due to alteration of ovarian activity (Hafez, 2000). During estrus, the main driving force is estradiol  $17\beta$  along with other estrogens which maintains the functional activity of female sex organ as well as for induction of sexual activity. The full expression of behavioural estrus or standing estrus in females could be induced by estrogen alone; however, progesterone and estrogen balance is physiologically required for exhibition of overt estrus. In the present context, the estrus behaviour rating is in partial confirmation with Rao and Rao (1981) who used overall 20 points for representing various external behavioural signs during estrus with the cows being graded into 3 categories as per the expression of sexual behaviors (weak heat < 7 points, normal heat 8-14 points and intense heat > 14 points). Such type of application will be practical in evaluating estrus signs more comprehensively. In this study, the Group I and Group II cows showed a higher

conception rate which were administered with progesterone (P-Depot) along with estradiol valerate (Progynon -depot) with the aim of inducing synchronous emergence of a new ovarian dominant follicle followed by estrus exhibition and ovulation. Administration of estradiol along with progesterone is beneficial due to its luteolytic property. In cows administration of estrogens can induce a preovulatory like LH surge and ovulation (Lammoglia *et al.*, 1998). Estrogens have been seen to induce follicular atresia (Hutz *et al.*, 1988) and the positive feedback effects of estrogens on gonadotrophins and preovulatory follicles have been reported in several studies. The overall conception rate observed in this study is at par with the findings of Costa *et al.*, (1993), Andurkar and Kadu (1995) and Narendra Kumar *et al.*, (2000). The progesterone and estradiol combination therapy in comparison to progesterone alone was proved to have higher overall conception rate in postpartum anoestrus cows which might be due to synchronous and synergistic effect of progesterone and estradiol on reproduction process.

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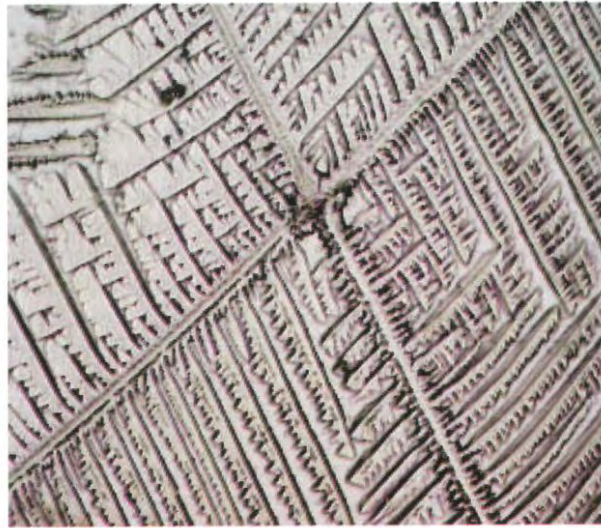


Fig. TYPICAL FERN PATTERN OF MUCUS OF INDUCED POSTPARTUM ANESTRUS COWS

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