CORRELATION BETWEEN SERUM STEROID HORMONE PROFILES BEFORE, DURING AND AFTER NORGESTOMET INDUCED OESTRUS AND OCCURENCE OF CONCEPTION IN REPEAT BREEDER CROSSBRED COWS*

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Abstract

A total of 16 crossbred repeat breeder cows were treated with norgestomet (Syncromate-B) ear implants for nine days. At the time of implant insertion, two ml of SMB injection was adminisetred to all the cows intramuscularly on day 10 of the oestrous cycle to induce oestrus. AI was done at 48 and 72 h after implant removal. Pregnancy diagnosis was done at 60 days following AI after induced oestrus to assess the first service conception rate and it was found to be 43.75 per cent. Blood samples were collected from all the cows at different stages following norgestomet treatment for oestradiol and progesterone assays. The mean progesterone level (ng/ml) and oestrogen levels (pg/ml) at the time of natural oestrus, day of initiation of oestrus induction treatment, implant removal and at first AI and at second, fourth and sixth day following first AI in cows which became pregnant (n=7) was 0.36±0.08, 7.19±0.46, 1.45±0.24,0.18±0.05, 1.05±0.20, 1.70±0.22 and 3.37±0.77 and 38.83±12.24, 11.33±3.54, 43.65±17.05, 20.63±3.44, 10.37±6.07, 9.68±3.71 and 14.28±4.77 respectively and the corresponding values in cows which did not conceive (n=9) at induced oestrus were 0.51±0.09, 5.24±0.43, 1.29±0.20, 0.23±0.03, 0.57±0.15, 0.99±0.27 and 1.62±0.26 and 15.13±5.14, 19.57±5.50, 25.86±13.54, 19.11±4.36, 23.51±5.35, 24.39±6.60 and 20.40±6.41, respectively. It is concluded that serum progesterone and oestrogen levels at various stages following syncromate –B therapy influenced the conception rate in repeat breeder cows.

Key words: *Norgestomet, Syncromate –B, Oestrus, Repeat breeder cows*

Since serum progesterone concentration directly reflected the function of corpus luteum, it was considered as an indicator of ovarian function and hence, could be used as a marker to predict the response to hormone treatment (Bulman and Lamming,1978). Administration of exogenous hormones to induce oestrus might change the endocrine status of treated animals (Selvaraju,1997). Hence, estimation of hormones before, during and after oestrus induction treatment might be helpful in predicting the nature of ovarian function and thus response to treatment. Roberts (1986) suggested that more investigations should be done with regard to the progesterone levels before, during and a few days after oestrus as a cause of the repeat breeder syndrome in cattle. Randel *et al*. (1971) noted that repeat breeder cows had altered serum estrogen levels during the first nine days after insemination compared to normal cows. Based on these views, the present investigation was undertaken to study the relationship of serum

** Part of Ph.D thesis submitted by the first author to the Tamil Nadu Veterinary & Animal Sciences University, Chennai-51*

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progesterone and oestrogen profiles before, during and after norgestomet induced oestrus and establishment of pregnancy in repeat breeder crossbred cows.

Materials and Methods

Healthy, parous crossbred cows which failed to conceive after three or more AIs were subjected to thorough gynaecolgical examination and cows free from any gross palpable abnormalities and obvious infections of the genital tract were included in this study. A total of 16 crossbred repeat breeder cows were used. Sexual rest was given during one oestrus following selection. Norgestomet ear implants (Syncromate-B, SMB system, Sanofi, Animal Health Inc., U.S.A) containing 6 mg of synthetic progesterone (norgestomet) were inserted aseptically and subcutaneously in the middle third of the outer surface of the pinna of the ear of all cows using an applicator on day 10 following natural oestrus. At the time of ear implant insertion, 2 ml of SMB injection (Sanofi, Animal Health Inc., U.S.A) containing 5 mg oestradiol valerate and 3 mg norgestomet was administered intramuscularly to all the cows. AI was done at 48 and 72 h of implant removal. Pregnancy diagnosis was done at 60 days following AI at induced oestrus and first service conception rate was calculated (number of cows conceived divided by number of cows treated and expressed in percentage). Blood samples were collected from all the cows at natural oestrus, at the time of implant insertion, implant removal, at the time of first AI at induced oestrus and at second, fourth and sixth day following first AI for oestradiol and progesterone assay. Serum was separated by centrifuging the clotted blood at 3000 rpm for 10 min. The serum samples were stored at -80°C for progesterone and oestradiol assay. Serum progesterone and oestradiol assays were carried out using progesterone RIA kit (PROG- CTK – 4; DiaSorin, s.r.l. Saluggia (vc), Italy) and oestradiol RIA kit (ESTR- CTK–4; DiaSorin, s.r.l.Saluggia (vc), Italy) respectively by employing solid phase Radioimmunoassay technique. The radio activity was measured in I^{125} gamma counter. The mean serum progesterone and oestradiol concentrations at different stages following oestrus induction were correlated with the conception in repeat breeder cows.

Results and Discussion

In the present investigation, cent per cent oestrus response was obtained following implant removal. This was in agreement with the findings of Odde (1990) in norgestomet and oestradiol valerate treated cows. The effectiveness of norgestomet in this study might be attributed to the combined effects of progestagen priming on the brain and the direct effect on the hypothalamus by both exogenously administered oestrogen and the high concentration of oestrogen that occurred in association with use of norgestomet-oestradiol (Cavalieri and Fitzpatrick, 1995). Following norgestomet treatment, the first service conception rate obtained was 43.75 per cent in this study. This was more or less similar to the conception rate obtained in cows in an earlier study with norgestomet (Hixon *et al.,*1981). Many investigators recorded that the first service conception rate ranged from 33 to 68 per cent in norgestomet treated cows (Odde, 1990; Cavalieri and Fitzpatrick, 1995). However, a very low conception rate of 18.20 per cent was also reported in norgestomet treated cows (Rentfrow *et al.,* 1987).

The mean progesterone level (ng/ml) at the time of natural oestrus, day of initiation of oestrus induction treatment, implant removal at first AI and at second, fourth and sixth day following first AI in cows which became pregnant (n=7) at induced oestrus was 0.36±0.08, 7.19±0.46, 1.45±0.24, 0.18±0.05, 1.05±0.20, 1.70±0.22 and 3.37±0.77 respectively and the corresponding values in cows which did not conceive (n=9) at induced oestrus were 0.51±0.09, 5.24±0.43, 1.29±0.20, 0.23±0.03, 0.57±0.15, 0.99±0.27 and 1.62±0.26 respectively.

In this study, the level of progesterone at natural oestrus and induced oestrus was around or less than 0.5ng/ml in cows which conceived and in those which did not conceive. The level progesterone observed at natural and induced oestrus in these cows was in accordance with the finding of Agarwal *et al.* (1989) in repeat breeder cows. In the present study, the marginal reduction in progesterone level was noticed in induced oestrus when compared to natural oestrus both in cows which became pregnant and in cows which did not conceive after AI at induced oestrus. In accordance with this finding, Gupta *et al*. (1998) found that the mean serum progesterone level was lower on the day of oestrus in cows which conceived (0.29 ng/ml) than in those which did not (0.32). Duchens *et al.* (1995) suggested that elevated progesterone level after luteolysis might lead to asynchrony between the onset of oestrus and ovulation and consequently a cause of repeat breeding in cattle. Similarly, Agarwal *et*

al. (1989) stated that 62 per cent of repeat breeders had abnormal progesterone secretory pattern during oestrus.

The higher concentration of progesterone observed on the day of oestrus induction (day 10 of the previous cycle) in pregnant cows (7.19±0.46) compared to non pregnant cows (5.24±0.43 ng/ml) was in accordance with the finding of Breuel *et al*. (1989). Folman *et al.* (1973) reported that the progesterone level during the oestrous cycle preceeding insemination was closely related to the occurrence of conception. The level of progesterone (6.31±0.32) noticed at the time of initiation of treatment in animals studied was significantly reduced to a lower level (1.38±0.16 ng/ml) by the time of implant removal in pregnant and non-pregnant cows. Similar trend was also noticed in previous studies in norgestomet treated cows (Garverick *et al*., 1988; Cavalieri and Fitzpatrick, 1995). Further none of the cows in this study exhibited oestrus during the implant period. Norgestomet was found to have approximately 300 folds more biological activity than natural progesterone in the dairy cows (Barnes *et al*., 1981). This might be the reason for the effective control of oestrus during the implant period in norgestomet treated cows in this experiment.

In the present study, both natural oestrus and induced oestrus in pregnant cows showed marginally lower progesterone profile when compared to non-pregnant cows. This was in agreement with the finding of Gustafsson *et al.*(1986). Higher progesterone level at the time of oestrus might affect sperm and ovum transport as well as the fertilization process and subsequent embryo passage to the uterus (De Silva *et al.,*1981). Anderson and Day (1994) opined that increased progesterone level blocked the LH release and affected oocyte maturation and ovulation. Further, Duchens *et al.* (1995) stated that suprabasal progesterone level delayed the ovulation and lead to retention of Graafian follicle for an extended period and damage of the oocyte to such an extent that even inseminating close to the time of ovulation did not ensure conception. Thus the marginally higher concentration of progesterone at natural and induced oestrus recorded in non-pregnant cows might have been one of the contributing factors for the failure of conception in this study.

In the present investigation, from day 2 to 6 post AI, the concentration of progesterone was higher in pregnant cows than in non-pregnant cows. Further, the difference was statistically significant ($P<0.01$) on day 6 after AI. This was in agreement with the finding of other study on repeat breeder cows (Bugalia and Sharma, 1990). Many investigators studied the progesterone concentrations in pregnant and non-pergnant cows to determine whether these affected the fertility. Duchens *et al.* (1995) found a higher plasma progesterone concentration in pregnant cows between day 10 and 18 than in non-pregnant females. Peters (1996) suggested that progesterone secretion could be a limiting factor to embryonic development during the first few days of pregnancy in bovines. The more rapid increase in progesterone level from day 2 to 6 post insemination in pregnant cows compared to non-pregnant cows in this study indicated a higher level of luteal activity which might have resulted in conception.

In this experiment, the mean serum oestradiol levels (pg/ml) at the time of natural oestrus, day of initiation of treatment (day 10 of previous oestrous cycle), implant withdrawal, day of first AI and at second, fourth and sixth day after first AI were 38.83±12.24, 11.33±3.54, 43.65±17.05, 20.63±3.44, 10.37±6.07, 9.68±3.71 and 14.28±4.77 in pregnant cows and 15.13±5.14, 19.57±5.50, 25.86±13.54, 19.11±4.36, 23.51±5.35, 24.39±6.60 and 20.40±6.41 in non-pregnant cows, respectively. The mean levels of oestradiol recorded in this study at natural oestrus in cows which conceived (38.83 ±12.24) and those did not conceive (15.13 ±5.14 pg/ml) was in accordance with the levels reported by Coe and Allrich (1989). However, a lower serum oestradiol level (8 to 10 pg/ml) at natural oestrus was reported in repeat breeder cows (Gupta *et al.,* 1998). The levels of serum oestradiol measured at induced oestrus in this study in pregnant and non-pregnant cows (20.63±3.44 and 19.11±4.36 pg/ml, respectively) were in concurrence with the reported value of Bugalia and Sharma (1990) in repeat breeder cows. Maurer and Echternkamp (1985) reported a lower oestradiol concentration (8 to 10 pg/ml) in repeat breeder cows.

In the present study, the levels of oestradiol on day 2, 4 and 6 after AI were lower in cows which conceived than in those that had not conceived. Similar finding was made by Lukaszewska and Hansel (1980). However, Agarwal *et al*. (1989) found significantly higher oestradiol levels in cows which conceived to

service than those cases reported again. Further, Maurer and Echternkamp (1985) compared the oestrogen levels between control and repeat breeder cows during oestrous cycle and found that repeat breeder cows had lower oestrogen profile when compared to control. There was no significant (P>0.05) difference in the oestradiol levels between cows which became pregnant and those which did not conceive at the induced heat on all the days measured in this study. This study might be attributed to variations of the individual cows in the secretory pattern of oestradiol during oestrus induction and the ensuing period.

Hence, from this study it is concluded that lower level of serum progesterone during natural, induced oestrus and implant removal and higher level of progesterone on the day of oestrus induction, and elevated levels of progesterone during early luteal phase ensured conception in repeat breeder cows. Further, increased concentration of serum oestrogen during natural oestrus, induced oestrus and implant removal and lower concentrations on day 10 of the preceeding cycle and during early luteal phase determined conception in repeat breeder cows.

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