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Effect of post-insemination progesterone supplementation on conception rate in repeat breeding cattle

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Establishment and maintenance of pregnancy in cattle is largely dependent on the ability of corpus luteum to secrete progesterone (Mann and Lamming 1995, Goff 2002). Delayed formation of the corpus luteum (CL) either alone or in combination with lowered secretion of progesterone during luteal phase has been attributed as one of the major causes of repeat breeding syndrome (Thatcher *et al.* 1994, Honparkhe *et al.* 2006). Normal early embryonic development and pregnancy were found associated with increasing progesterone activity initiated on days 4.5–10 post insemination (Albihin *et al.* 1991, Larson *et al.* 1997, Kasrija *et al.* 2006). Hence, the deficiency in progesterone concentration leads to early embryonic losses. Clinical trials in catle have been conducted to study the effect of progesterone supplementation on pregnancy rates ended with conflicting results (Robinson *et al.* 1989, Van Cleeff *et al.* 1989). Therefore, the study was planned to observe the effect of post-insemination progesterone supplementation on pregnancy rates in repeat breeding crossbred cattle.

Repeat breeding dairy cattle (30) in their second to fourth parity, each weighing between 300–550 kg, kept under semi-loose type housing system were selected. All selected animals had normal genital organs, normal estrous cycle, and apparently clear cervico-vaginal discharge but had failed to become pregnant after 4–8 consecutive inseminations with fertile semen. The cows were divided in to 2 groups consisting of 20 in group 1 (untreated cows) and 10 in group 2 (supplemented with 500 mg of 17–α hydroxy progesterone caproate intramuscularly on 5th and 11th days post-insemination).

Estrus detection of all the cows was done twice daily by vasectomized teaser bull. The cervico-vaginal mucus was also examined microscopically for fern pattern. All the animals were inseminated at standing estrus (day 0) with good quality frozen thawed semen. All the animals were subjected to per-rectal examination on days 60–90 post-insemination for confirmation of pregnancy. Another criteria i.e. skim milk $P_4 > 1.5$ ng/ml on day 22 post-insemination was also considered for pregnancy confirmation.

Milk samples (7) were collected (with sodium azide @ 100 mg/ 10 ml of milk, as a preservative) from each animal on days 0, 4, 6, 8, 12, 16, 20 or 22 post- insemination and centrifuged at 2000 G for 15 min for P_4 estimation. The centrifuged samples were kept in refrigerator for 30–45 min and stored in deep freezer (–20°C) after removal of fat layer. P_4 was estimated by radioimmunoassay precoated tube method with the help of kits. The sensitivity of RIA was 0.028 ng/ml. The mean intra- and inter-assay coefficient of variation were 11.2 and 8.9, respectively. The data were analyzed statistically according to student's t-test using difference between 2 means application (Gupta 1997).

Out of 20 repeat breeder untreated cow (group 1), only 4 animals became pregnant. The conception rate was 20%. The remaining 16 cows returned to estrus after 20–22 days post-insemination. Lower conception rate in untreated repeat breeding cows could be due to fertilization failure or early embryonic death (Kimura *et al.* 1987, Humbolt 2002). In repeat breeding treated cows (group 2, n=10) only 3 cows became pregnant while 7 remained non-pregnant following P_4 supplementation. The conception rate (30%) in this group was 10% more than that of group 1. This indicated that supplementation of post-insemination P_4 had nonsignificantly increased the conception rate. Assessment of the effect of exogenous P_4 supplementation is extremely difficult (Peters 1996). Some workers reported significant improvement (Robinson *et al.* 1989, Umakanthan 1995) while others showed reduction/nonsignificant improvement (Diskin and Sreenan 1986, Van Cleeff *et al.* 1989) in conception rates.

Analysis of P_4 concentration in pregnant repeat breeder treated and untreated cows revealed that treated cows had significantly higher P_4 as compared to treated cows (day 16;

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3.03 vs 2.48 ng/ml and day 22; 3.7 vs 2.82 ng/ml). Higher progesterone concentrations in supplemented cows could be due to cumulative effect of progesterone supplementation. The non-pregnant untreated cows had lower progesterone on days 8 (1.05 vs 1.5 ng/ml) and 12 (1.40 vs 2.01 ng/ml) than non-pregnant treated ones, which might be due to progesterone supplementation on days 5 and 11 post-insemination. Sreenan and Diskin (1983) reported no effect of progesterone administration @ 100 mg daily between days 10 and 20 post-insemination on plasma progesterone level over this period. Similarly, Walton *et al.* (1990) observed nonsignificant effect of progesterone supplementation @ 200 mg on days 5, 7, 9 and 11 post-insemination on plasma levels. In present study, exogenous progesterone was unable to raise the endogenous progesterone profiles significantly. It is assumed that progesterone suppresses the pulse frequency of pituitary luteinizing (LH) hormone (Ireland and Roche 1982) and thereby depresses the circulating concentrations of LH (Roche and Ireland 1981), the major luteotropin of cattle. The exogenous progesterone might have suppressed LH and thereby suppressed the endogenous progesterone production by corpus luteum. Similar results were obtained by Robinson *et al.* (1989) in cows supplemented with PRID from day 10–17 post-insemination. However, the exogenous progesterone supplementation should result in increased skim milk progesterone concentration because progesterone crosses the mammary gland (Darling *et al.* 1972). It might be possible that the decrease in endogenous progesterone concentration was overcome by increase in skim milk progesterone concentration resulting nonsignificant effect of exogenous progesterone administration on skim milk progesterone levels.

SUMMARY

The study was planned to observe the effect of post-insemination progesterone supplementation on pregnancy rates in repeat breeding crossbred cattle. The supplementation of P_4 had increased the conception rate by 10% in treated animals. However, it failed to cause significant rise of milk P_4 concentration in these animals as compared to control ones. The extensive studies are required regarding P_4 supplementation in relation to days post AI to assess its significance.

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