Title of thesis: Studies on clinical efficacy of some therapeutic regimen(s) on conception rate in repeat breeder cows showing metestrual bleeding

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SUMMARY

The objectives of this study was to record the overall incidence of metestrual bleeding in the cows, to assess the percentage of repeat breeder cows showing this malady and to ascertain and suggest the remedial measure for this malady.

The data of 10713 crossbred cows from 10 districts of Himachal Pradesh was collected from doctors, pharmacists and farmers in different clinical camps and through a questionnaire distributed in different field veterinary institutions to record the overall incidence of metestrual bleeding and associated repeat breeding.

To suggest the possible therapeutic measures, 447 crossbred cows presented for insemination in Veterinary clinical complex of Dr. G.C Negi College of Veterinary and Animal Sciences, Palampur and nearby Veterinary hospitals were included in the study. This included 373 repeat breeders animals that were free from any reproductive disorder, except metestrual bleeding in previous cycles, and not conceiving and 74 normal cows without bleeding.

Animals selected for the study were distributed in three treatment (viz. progesterone, Busereline acetate and Lugol's Iodine) and two control (untreated and normal control) groups. The animals included in progesterone and Busereline acetate treatment groups were subdivided into three groups each viz. the hormones administration simultaneous to AI (group 1 and 4), insemination and exogenous administration of hormones on 3rd day post AI (group 2 and 5) and insemination and administration of hormones on 5th day post A.I (group 3 and 6), respectively. Thirty animals with the history of metestrual bleeding were treated with 0.1 per cent Lugol's Iodine 30 ml infusion intrauterine, for consecutive three days and insemination was done in subsequent estrus. Thirty one crossbred cows exhibiting metestrual bleeding were inseminated without any treatment as untreated control. Seventy four crossbred cows without any metestrual bleeding were kept as normal control. The insemination was done in standing heat without any treatment.

Artificial insemination was performed with frozen thawed semen either once or twice. In all the double insemination groups, second AI was performed about 24 hours later. Irrespective of single or double insemination, day of first insemination was considered as day 0 for
progesterone or Busereline acetate treatments. Pregnancy diagnosis was carried out after 60 days of AI by rectal palpation in animals not returning to estrus.

Nine cows showing metestral bleeding and 6 without bleeding were selected for collection of blood samples for progesterone estimation. Four samples, respectively, from each cow were collected on day 0, 3, 5 and 7.

**Incidence of metestral bleeding**

Overall, 10713 animals were surveyed in ten districts of Himachal Pradesh, out of which 1292 (12.06%) cows were reported to be showing metestral bleeding. In districts Kangra, Hamirpur, Mandi and Chamba 10.14, 13.71, 11.67 and 10.86 per cent cows showed metestral bleeding, respectively. Similarly, in district Shimla, 16.12 per cent, in district Solan 15.39 per cent and in district Una 22.67 per cent cows were exhibiting metestral bleeding. In districts Sirmour, Kullu and Bilaspur, the percentages of cows exhibiting metestral bleeding were 11.82, 17.68 and 14.35 per cent, respectively.

The overall incidence of metestral bleeding was more in heifers (13.91%) as compared to pluriparous cows (11.49%). In district Kangra, the incidence of metestral bleeding was 14.29 per cent in heifers as compared to 8.71 per cent in pluriparous cows. In district Hamirpur the incidence of bleeding was 21.95 and 11.89 per cent and in district Mandi it was 11.2 and 11.84 per cent in heifers and pluriparous cows, respectively. Similarly, in district Chamba, 14.70 per cent heifers and 10.16 per cent pluriparous cows were showing metestral bleeding where as in district Shimla 17.64 per cent heifers and 15.63 per cent calved animals exhibited this condition. In districts Solan, Una, Sirmour and Kullu 20.00, 26.83, 12.42 and 17.29 per cent heifers and 13.51, 21.37, 11.69 and 17.78 per cent pluriparous cows, respectively, were observed showing metestral bleeding. In district Bilaspur, the incidence of metestral bleeding was 19.75 per cent in heifers and 13.13 percent in pluriparous cows.

**Incidence of repeat breeding associated with metestral bleeding**

Overall, out of 1292 cows reported to be showing metestral bleeding in ten districts of Himachal Pradesh 1023 (79.18%) animals were repeat breeder. In district Kangra 80.24, in district Hamirpur, 83.70 and in district Mandi 77.43 per cent cows were reported to be having repeated conception failure consequent upon metestral bleeding. Similarly, in districts Chamba, Shimla, Solan and Una 75.0, 72.16 62.50 and 84.62 per cent, respectively, were observed as repeat breeder cows due to this malady. In districts Sirmour, Kullu and Bilaspur 83.33, 79.05 and 82.54 per cent cows, respectively, had repeated conception failure associated with metestral bleeding.

Amongst animals having metestral bleeding, overall, comparatively fewer heifers (75.93 %) than cows (80.38 %) were repeat breeders. In district Kangra, the incidence of repeat breeding within the animals showing metestral bleeding was 79.34 and 80.75 per cent
in heifers and pluriparous cows, respectively. Similarly, 77.77 per cent heifers and 86.36 per cent calved cattle in Hamirpur and 69.15 per cent heifers and 80.14 per cent pluriparous cows in district Mandi were repeat breeder amongst animals with metestrual bleeding. Likewise, 73.34 per cent heifers and 75.44 per cent pluriparous cows had repeat breeding associated with metestrual bleeding in district Chamba. The incidence of repeat breeding with metestrual bleeding in heifers and pluriparous cows was 71.43 and 72.42 per cent, 66.66 and 60.00 per cent, 81.81 and 85.72 per cent and 80.56 and 84.03 per cent in districts Shimla, Solan, Una and Sirmour, respectively. In district Kullu 78.26 and 79.27 per cent incidence of repeat breeding incidental to metestrual bleeding was reported in heifers and pluriparous cows, respectively, and it was 81.25 and 82.97 per cent in heifers and calved cows, respectively, in district Bilaspur.

Clinical efficacy of different treatments on conception rate in repeat breeder cows showing metestrual bleeding

In first group, in which 43 cows showing metestrual bleeding were administered Hydroxy progesterone caproate intramuscularly simultaneous to A.I. (day 0), 13 conceived with an overall conception rate (CR) of 30.23 per cent. In second group, among 95 cows injected progesterone third day post AI, 48 conceived with a CR of 50.53 per cent and in third group, 32 out of 68 (47.06%) inseminated were pregnant following intramuscular progesterone administration on fifth day post insemination.

In the groups 4th, 5th and 6th where Busereline acetate was administered intramuscularly to 37, 38 and 31 cows, on day 0, 3 and 5 post AI, 12 (32.43%), 11 (28.94%) and 9 (29.03) cows, respectively, conceived.

In seventh treatment group comprising of 30 cows administered Lugol’s Iodine intrauterine in one cycle and inseminated in subsequent estrus, 8 (26.67 %) conceived. In untreated control cows (group 8), 31 animals showing metestrual bleeding were inseminated without any treatment, of which 8 (25.80%) conceived. Amongst normal cows (n=74) without any metestrual bleeding (group 9), 39 (52.70%) conceived.

Insemination of 24 and 19 cows in group 1 with the administration of Hydroxy progesterone caproate, simultaneous to A.I., resulted in conception of 7 (29.17%) and 6 (31.58%) animals following single or double insemination, respectively. Similarly, following the insemination of 52 and 43 cows, injected progesterone third day post AI (group 2), 23 (44.23%) and 25 (58.14%) animals conceived following single or double insemination, respectively. In group 3, of the 37 animals, 18 (48.65%) conceived with single and out of 31 cows 14 (45.16%) conceived following double A.I. after the administration of progesterone 5th day post insemination in cows showing metestrual bleeding.
After the insemination of 19 and 18 cows (group 4) along with administration of Busereline acetate simultaneous to A.I., 5 (26.32%) and 7 (38.89%), following the insemination of 19 cows in each of the two groups injected Busereline acetate third day post AI (group 5), 4 (21.05%) and 7 (36.84%) and following the insemination of 17 and 14 cows, injected hormone fifth day post AI (group 6), 5 (29.42) and 4 (28.57) animals conceived following single or double insemination, respectively.

In Lugol’s Iodine treatment group (group 7), 4 (22.23%) cows conceived out of total 18 inseminated following single and 4 (33.34%) out of 12 became pregnant following double insemination. Among untreated control animals (group 8), 5 (25.00%) and 3 (27.28%) cows conceived out of 20 and 11 cows inseminated after single or double insemination, respectively. Amongst normal control cows (group 9), 26 out of 51 (50.98%) conceived with single insemination and 13 of 23 (56.52%) became pregnant after double inseminations at 24 hours interval, respectively.

Insemination of 19 heifers and 24 pluriparous cows in group 1 along with intramuscular administration of Hydroxy progesterone caproate simultaneous to A.I., resulted in conception of 6 (31.58%) and 7 (29.17%) animals, respectively. Similarly, following the insemination of 36 heifers and 59 pluriparous cows, injected progesterone third day post AI (group 2), 20 and 28 animals conceived with a CR of 55.56 and 47.46 per cent, respectively. In group 3, of the 33 heifers, 15 (45.46%), and out of 35 pluriparous cows 17 (48.57%) conceived after the administration of progesterone 5\textsuperscript{th} day post insemination in cows showing metestrual bleeding.

Insemination of 17 heifers and 20 pluriparous cows in group 4 along with the administration of Busereline acetate intramuscularly simultaneous to A.I., resulted in conception in 5 (29.41%) and 7 (35.00%) animals, respectively. Similarly, following the insemination of 13 heifers and 25 pluriparous cows, injected Busereline acetate third day post AI (group 5), 4 and 7 animals conceived with a CR of 30.77 and 28.00 per cent, respectively. In group 6, out of 12 heifers, 3 (25.00%), and out of 19 pluriparous cows 6 (31.58%) conceived after the administration of Busereline acetate 5\textsuperscript{th} day post insemination in animals showing metestrual bleeding.

In Lugol’s Iodine treatment group (group 7), 4 (28.57%) heifers and 4 (25.00%) pluriparous cows conceived out of total 14 and 16 inseminated, respectively. Among untreated control animals (group 8), 3 (37.50%) out of 8 inseminated heifers conceived, whereas, only 5 (21.74%) pluriparous cows conceived from among 23 inseminations. Amongst normal control cows (group 9), 10 out of 21 (47.62%) heifers and 29 of 53 (54.71%) pluriparous cows, respectively, became pregnant.

Overall CRs in heifers and cows showing metestrual bleeding was 39.48 and 36.65 per cent, respectively.
Irrespective of parity, 34.47 and 41.92 per cent animals showing metestrual bleeding became pregnant following single or double insemination, respectively.

**Plasma progesterone concentration in cows exhibiting and not exhibiting metestrual bleeding**

Mean plasma progesterone concentration on day 0 of the estrus was 0.67±0.05 ng/ml in the cows showing metestrual bleeding and 0.64± 0.06 ng/ml in cows with no metestrual bleeding. On day 3, this value was 0.86± 0.03 and 0.92± 0.05 ng/ml in the cows with and without metestrual bleeding, respectively. Mean plasma progesterone concentration increased to 1.60±0.13 and 1.14±0.12 ng/ml on day 5 and 1.68±0.22 and 1.39±0.18 ng/ml on day 7 in the cows with and without metestrual bleeding, respectively.

**The conclusions drawn from this study are;**

1. The overall incidence of metestrual bleeding was 12.06 per cent in crossbred cows of Himachal Pradesh. Out of these 79.18 per cent were repeat breeders due to this malady.
2. Incidence of metestrual bleeding was more in heifers as compared to pluriparous cows, however, less heifers were repeat breeders (75.93%) as compared to calved animals (80.38 %).
3. Clinically, progesterone injected 3rd day post AI in cows with metestrual bleeding was the most efficacious. Progesterone injected 5th day post AI was equally effective. Other treatments viz; progesterone administration simultaneous to AI, Busereline acetate administration simultaneous to AI or 3rd or 5th day post AI or intra uterine infusion of Lugol's Iodine were not effective in treating repeat breeding due to metestrual bleeding.
4. Irrespectively of the treatment, the overall CR was better in heifers as compared to pluriparous cows showing metestrual bleeding.
5. Conception with double insemination at an interval of 24 hours was better as compared to single AI.
6. Although, progesterone injected 3rd or 5th day post AI improved conception significantly, the cows showing metestrual bleeding did not appear to be deficient in plasma progesterone concentration in comparison to normal cyclic cows.