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Estrus and fertility response of postpartum anestrus Murrah buffaloes to Crestar and OvSynch treatment regimens

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Buffalo reproduction is affected by long postpartum anestrus periods with poor estrus expression, further compounded by low conception rates to artificial inseminations. As a result, not only the life-time production declines, but the net calf crop is also reduced leading to economic losses to farmers- majority of whom themselves remain oblivious to the situation. Several therapeutic protocols were used for mimicking the endocrine events culminating into spontaneous estrus followed by ovulation (Barile 2005). In anestrus buffaloes, estrus was induced following treatment with Crestar (Hattab *et al*. 2000, Singh *et al*. 2004), Syncromate B (Misra *et al.* 2003) and OvSynch (Sharma *et al.* 2004), though the results remain inconclusive. Therefore, further work on application of hormonal therapies for treatment of true postpartum anestrus buffaloes is warranted, hence the present study was undertaken.

Animals and experimental design: The experiment was conducted from August to November in primiparous Murrah buffaloes (n=15) from the institutional herd. These buffaloes were clinically anestrus for 180 to 324 days postpartum. Anestrus status was confirmed by the absence of a corpus luteum in either of the 2 ovaries by ultrasonographic monitoring, twice at an interval of 10 days. Basal peripheral plasma progesterone concentrations (<0.50ng/ml) further validated diagnosis of a true anestrus status.

These suckled buffaloes were kept under standard loose-house farm management practice and randomly divided into 2 groups to receive either Crestar implant (Crestar group, n=7, age=60.0±1.8) or OvSynch therapy (OvSynch group, n=8, age=59.1±1.6). Mean postpartum interval at the start of treatment was 234.3±22.5 and 262.5±15.7 days in Crestar and OvSynch groups, respectively.

Treatment protocols: Crestar group animals received subcutaneous ear implant containing 3 mg norgestomet with an intramuscular injection of 3 mg norgestomet and 5 mg estradiol valerate on day 0 (day of start of experiment). Additionally 500 IU eCG was administered intramuscularly on day 9. On day 10, the implant was removed while on day 12 another intramuscular injection of 16 µg GnRH analogue was administered, followed by frozen semen insemination at detected estrus.

The OvSynch protocol (OvSynch group) consisted of 500 IU eCG (Folligon) on day 0, 16 µg GnRH analogue (first GnRH) on day 3, 25 mg $PGF_2\alpha$ analogue on day 10 and 16 µg GnRH again on day 12 (second GnRH), followed by fixed time AI at 6 and 30 h after second GnRH.

Estrus detection, AI and PD: Estrus detection was done by teaser bull paraded twice daily and confirmed by per-rectal palpation and ultrasonography of genitalia and ovaries at the time of AI with frozen semen. Daily ultrasound examinations were done to record ovulations. Follicle was identified as a non-echogenic structure and its development/ regression was monitored with sequential ultrasonography. Ovulation was detected by sudden disappearance of a dominant follicle and confirmed by the development of a corpus luteum at the same location on subsequent ultrasonographic examinations 3–4 days later. Corpus luteum area (CLA) was determined by measuring the maximum diameter of the corpus luteum at two angles and then deriving CLA from the equation (Kastelic *et al.* 1990) :

CLA in mm^2 = (L×0.5)×(W×0.5)×3.14

Animals returning to estrus were re-inseminated till conception to determine the number of inseminations per conception and overall interval from treatment to conception.

Pregnancy diagnosis was also done by trans-rectal ultrasonography on day 30 post insemination and was considered positive when non-echogenic fluid in the uterus or an echogenic embryo surrounded by fetal fluids was

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observed. Palpation per rectum was done to confirm the gravidity around 80 days post AI.

The results were subjected to statistical analysis (Snedecor and Cochran 1989). Student's t-test was used for comparing 2 variables and Duncan's Multiple Range Test for more than 2 variables.

All animals in the present study had calved normally and the uterine involution was completed uneventfully by day 40 postpartum as observed by per rectal ultrasonography. There was no history of uterine pathology.

Estrus response and ovulation: Estrus was induced in all Crestar group buffaloes (7/7, 100%) with a mean (±SE) interval of 2.86±0.13 days from treatment to ovulation. In OvSynch group, estrus was induced in 7/8 buffaloes (87.5%) with a mean post-treatment interval of 1.29±0.36 days to ovulation (Table 1). Interval from treatment to ovulation was significantly different (P<0.05) between the 2 groups. Commensurate with the incidence of estrus, corpus luteum (CL) was also detected in 7/7 (100%) and 7/8 (87.5%) animals of Crestar and OvSynch group, respectively, indicating induction of ovulatory estrus in both groups. The mean corpus luteum area (CLA) at 10 days post-ovulation did not differ between the 2 groups (Table 1).

Conception rate and restoration of ovarian cyclicity: Majority of the Crestar group animals (5/7 i.e. 71.4%) conceived to inseminations at the treatment induced estrus at mean interval of 6.0±4.6 days from treatment (GnRH inj.). Significantly (P<0.05) less animals (1/8, 12.5%) conceived at OvSynch induced estrus with significantly longer treatment to conception interval (44.2±11.7 days P<0.05). Up to third insemination, 6 animals in each group had become pregnant with overall pregnancy rates of 85.7 and 75%, respectively, in Crestar and OvSynch groups. Further, the Crestar treated animals required comparatively lower number of inseminations per conception (1.17) than OvSynch treated buffaloes (2.0). Consequently the mean (±SE) postpartum interval to conception in OvSynch group (332.8±23.4 days) was significantly higher than Crestar group (238.0±23.6 days). It may be clarified here that the postpartum interval to conception (238 days) is shorter than the interval to the end of the treatment (246 days) in Crestar group, as one animal of this group that did not conceive had postpartum interval of 320 days at the start of treatment. Therefore, when it is excluded from conception data, the mean value is lower.

Cyclicity was reestablished in all treated non-pregnant buffaloes of the 2 groups, however, interval from induced estrus to next estrus was long. The size of dominant follicle (DF) at day 0, location of the DF developing during treatment, animals depicting behavioural estrus symptoms (cervical mucus discharge and uterine tone) and animals showing abnormal estrus after treatment are presented in Table 2.

On comparing the efficiency of Crestar and OvSynch for treatment of postpartum anestrus zebu cows, Baruselli *et al*. (2002) reported higher pregnancy rates with Crestar (42.7%) than OvSynch (15%). The results of the present study in buffaloes are in close agreement with this finding as pregnancy rates of 12.5% at induced estrus were obtained with OvSynch, though higher pregnancy rates (71.4%) were achieved with Crestar. Our results are in consonance with those of Ghuman *et al.* (2008) who reported conception rate of 18% with OvSynch in true anestrus buffalo heifers. Conversely, Neglia *et al*. (2003) reported higher pregnancy rates with OvSynch treatment as compared to PRID treated buffaloes.

Estrus behavioural signs of mucus discharge and uterine tone were observed (Table 2) more in Crestar treated buffaloes (71.4%) than OvSynch group (37.4%). Malik *et al*. (2009) reported that 6/15 (40%) buffaloes subjected to OvSynch treatment responded with good mucus discharge

Table 1. Ovulating follicle profiles (mean±SE) in Crestar and OvSynch treated anestrus buffaloes

Ovulating follicle profiles	Crestar group (n=7)	OvSynch group (n=8)
No. of animals ovulated	7	7
Day of ovulation (day 0= start of treatment)	12.86±0.13	13.0±0.49
Maximum diameter (mm) of ovulatory follicle	11.73±0.19	11.74±0.55
Interval from end of treatment to ovulation (days)	2.86±0.13[a]	1.29±0.36[b]
Corpus luteum area (mm^2) at 10 days post AI	226.19±13.17	193.22±27.20

In a row, mean values with different superscripts (a, b) differ significantly (P<0.05).

Table 2. Reproductive traits of Crestar and OvSynch treated postpartum anestrus buffaloes

Characteristics (day 0 = start of treatment)	Crestar group (n=7)	OvSynch group (n=8)
Follicle > 10 mm on day 0	5 (71.4)	6 (75.0)
Follicle > 9 mm on day 0	6 (85.7)	7 (87.5)
Follicle > 8 mm on day 0	7 (100.0)	8 (100.0)
Location of dominant follicle (DF) developing during treatment with respect to DF at day 0		
Ipsilateral	4 (57.1)	4 (50.0)
Contralateral	3 (42.9)	4 (50.0)
Animals induced to estrus	7 (100.0)	7 (87.5)
Animals with vulvar swelling at 48 h post-treatment	6 (85.7)	1 (12.5)
Animals with good mucus discharge and uterine tone	5 (71.4)	3 (37.5)
Animals with early estrus	1 (14.3)	2 (25.0)
Animals with late estrus	-	2 (25.0)
Animals with long estrus (>2 days)	1 (14.3)	2 (25.0)

Figures in parenthesis indicate percentages.

and uterine tone. Better conception rates at induced estrus in Crestar group might be due to the addition of eCG at implant removal leading to more vigorous corpus luteum for better fertility as in beef cattle (Baruselli *et al*. 2004). Better conception rates with Crestar could also be attributed to additional administration of GnRH (48 h after implant removal) because providing a stimulus to induce LH surge after progestin exposure is important to optimize estrus induction (Day 2004). Low conception rates at induced estrus following OvSynch treatment could be because animals used in this study were truly acyclic and hence not primed with progesterone, at the start of experiment. A short (5 to 9 days) exposure of anestrus animals to progestin can fulfill requirements of a successful estrus induction program (Day 2004). Progestin exposure reduces the concentration of estradiol receptors in the hypothalamus, thereby weakening estradiol negative feedback effect on GnRH release, leading to increased secretion of LH (Day and Anderson 1998). Findings of De Rensis *et al*. (2005) and Mc Dougall *et al*. (2005) confirm the important role of progesterone in priming the follicles to respond to the OvSynch protocol in non-cyclic buffaloes and cows.

Overall, Crestar treatment of postpartum anestrus buffaloes led to fertility of 71.4% at induced estrus, but it was only 12.5% in OvSynch treated buffaloes. However, total pregnancy rates upto third insemination were comparable, being 85.7% and 75% in Crestar and OvSynch group animals, respectively.

In conclusion, our results indicated that Crestar treatment supplemented with eCG towards the end of treatment schedule is more effective in inducing estrus and high fertility at detected estrus in postpartum anestrus buffaloes, as compared to OvSynch treatment. The low conception rates at induced estrus in OvSynch treatment needs to be explored further with respect to importance of the luteal support from the first CL or through exogenous progesterone supplementation.

SUMMARY

Primiparous postpartum anestrus Murrah buffaloes (15) were randomly divided into 2 groups to receive either Crestar (n=7) and OvSynch treatment (n=8) to evaluate their efficacy for induction of estrus. Estrus was induced in 100% and 87.5% buffaloes in Crestar and OvSynch group, respectively, with mean interval to ovulation of 2.86±0.13 and 1.29±0.36 days. The postpartum interval to conception was also higher in OvSynch group animals (332.8±23.4 vs 238.0±23.6 days). A conception rate of 71.4% to first insemination at induced estrus was recorded in Crestar group, whereas it was only 12.5% in OvSynch treated animals though respective overall pregnancy rates up to third insemination were 85.7 and 75%. In conclusion, ovulatory estrus could be successfully induced following Crestar treatment yielding satisfactory conception rates at induced estrus.

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