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Research Article

EFFICACY OF DIFFERENT HORMONES AND MINERAL SUPPLEMENTATION IN OVARIAN REBOUND AND FERTILITY IN POST-PARTUM ANESTRUS CATTLE

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ABSTRACT

The present study was aimed to evaluate the effect of different therapeutic protocols on post-partum anestrus cattle. Twenty-four post-partum anestrus cows were selected and divided into four groups. Group A (n = 06) were fed ration containing 0% mineral mixture, Group B (n = 06) were fed ration containing 1% mineral mixture, Group C (n = 06) was treated with double PG protocol then AI with 2.5 ml GnRH, and Group D (n = 06) were fed ration containing 1% mineral mixture and also subjected for double PG protocol then AI with 2.5 ml GnRH. The estrus induction response was 33.33, 50.00, 66.66 and 100% with corresponding and conception rates of 33.33, 50, 66.66 and 83.33% in Group A, B, C, and D, respectively. All the three treatment protocols can be effectively used for induction of estrus with acceptable conception rate in postpartum anestrus cows. However, mineral mixture supplementation with double PG protocol results in comparatively better pregnancy rate.

Keywords: Anestrus, Estrus induction, Fertility response, Hormone, Mineral

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Anestrus is one of the major reproductive problems in cattle (Butani *et al.*, 2008). PGF2α is effective in treating postpartum anestrus by virtue of its luteolytic properties. Deficiency of energy intake (Ling *et al.*, 2007) and minerals affect the initiation of estrus. Minerals like calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals as well as affect certain enzyme system which in turns may affect reproductive efficiency (Ibtisham and Nawab, 2018). Insufficient minerals (calcium and phosphorus, copper, manganese, zinc, etc.) inhibit function of the genital organs (Ibtisham and Nawab, 2018). The study was aimed to investigate effect of different therapeutic protocols on estrus induction response and conception rate as well as hormonal and mineral profile in true anestrus cattle.

MATERIALS AND METHODS

The study was conducted on postpartum true anestrus cows (3-5 years) under field conditions in Rewa districts of Madhya Pradesh. The experimental cows were kept in an intensive housing management system. A proper health assessment, including vaccinations and deworming, was performed before study began. Diets for the control group were formulated to have the same crude protein and energy levels based on the body weight to make the diet an isocaloric and isonitrogenous one. The experimental cows were allocated in 4 groups (6 in each group); Group A cows were fed only ration without mineral mixture (as control), Group B cows were fed ration containing 1% mineral mixture, Group C were subjected for double PG protocol (Cloprostenol sodium 500 µg, i/m) 11 days apart followed by AI 48 later with GnRH (Buserelin acetate, 10 µg, i/m) and Group D were fed mineral mixture as in group B along with hormonal treatment as in Group C.

Blood samples were collected by jugular venipuncture on day 0, 7, 14, 21, 42 and 60 during feeding trial for assay serum progesterone (ELISA kits, Cayman chemicals, USA) and estrogen (RIA technique using diagnostic I125 kits, Immunotech, France). The major elements (calcium, phosphorus and magnesium) were estimated by colorimetric method by using standardized kits whereas trace elements (copper, cobalt, zinc selenium and iron) were estimated by Atomic Absorption Spectrophotometer.

The estrus detection was carried out twice daily (6 AM and 5 PM) by visual observations and confirmed by the cervico-vaginal discharge and presence of tonicity and turgidity in the reproductive tract during the per-rectal examination. As soon as animals were detected in estrus, they were inseminated with frozen semen after 12h of estrus. Pregnancy diagnoses in cows of all groups were performed on day 60 post-insemination by per-rectal examination.

The statistical analysis was done using Chi-square test and analysis of variance (Snedecor and Cochran, 1994) using IBM® SPSS software, version 22 statistical packages.

RESULTS AND DISCUSSION

Estrus Induction Response and Conception rate in anestrus cows: The estrus induction response was 33.33,

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50.00, 66.66, and 100% with corresponding conception rate of 33.33, 50.00, 66.66, and 83.33% in Group A, B, C and D, respectively. The best estrus induction response and conception rate was observed in Group D followed by Group C, B and A.

Serum Progesterone and Estrogen Profile: Serum Progesterone and Estrogen Profile (Mean±SE) were depicted in Table 1. In the treatment group C and D, progesterone level (ng/ml) varies significantly ($P \leq 0.05$) from day 14 to day 60. A similar progesterone level can be observed on days 14, 21 and 42, but it differs significantly ($P \leq 0.05$) from day 0, 7 and 60. As compared with Group A, serum progesterone levels of B, C, and D Groups were significantly higher. Numerically higher progesterone level was noted in group D as compared to group C cows. Furthermore, group D cows has highest conception rate.

Low serum P4 levels have been reported in acyclic bovines (Singh *et al.*, 1998) but Kerr *et al.* (1991) observed that in about 36% of clinical anoestrus heifers, P4 levels did not corroborate their non-cyclic status. In the present experiment, regardless of basal P4 levels, a sharp rise in these values was observed in all treated cows. On day 14, pregnant animals of all groups have higher progesterone concentration as compared to non-pregnant animal of the same group. The present findings corroborated well with those of Patel *et al.* (2014) in repeat breeding cows using similar treatment protocols.

Among treatment groups, the estrogen level (pg/ml) did not varied significantly ($P \leq 0.05$) at day 0 but varied significantly from day 7 to 42. A deficiency of the estrogen hormone and irregularity in its production are observed in anestrus animals. Present findings concurred well with previous reports (Dayanidhi *et al.*, 2016; Mondal *et al.*, 2019).

Serum Mineral Profiles: Level of calcium (mg/dl), phosphorus (mg/dl), magnesium (mg/dl), copper (µg/ml), cobalt (µg/ml), zinc (µg/ml), iron (µmol/ml) and selenium (ppm) have been depicted in Table 2.

Serum Calcium: The periodic values (i.e. at day 0, 21, 42 and 60) of calcium differed significantly ($P \leq 0.05$) in all treatment groups (B, C and D) but no significant difference was noted in control group A. However, no definite pattern was noticed among the groups. The present study is consistent with Akhtar *et al.* (2009) and Tewari *et al.* (2013). In present study, the better results were achieved when animals were supplemented with mineral mixture and therefore had higher Ca values than the control group. Lower serum calcium levels were observed in delayed pubertal anoestrus heifers than normal cyclic heifers (Singh *et al.*, 2005). Furthermore, Kumar *et al.* (2020) opined that calcium sensitizes tubular genitalia for action of hormones.

Serum Phosphorus: Unlike present observation higher and lower phosphorus levels were recorded by many workers in acyclic animals (Das *et al.*, 2002; Singh *et al.*, 2005).

Serum Magnesium and copper: The mean serum magnesium and copper level was slightly higher in group D compared to other groups and phosphorus level were within normal range and in close agreement with Sharma *et al.* (2007) and Tiwary *et al.* (2007). Comparable copper level was reported by Dutta *et al.* (2001a,b). Copper deficiency may also be considered responsible for the anoestrus conditions as this is a component of enzymes involved in steroidogenesis (Hidiroglou, 1979).

Serum Cobalt: The serum cobalt concentration did not differ significantly within and among the groups. Conversely, Vohra *et al.* (1995) and Tambe *et al.* (1996) noticed lower cobalt levels.

Serum Zinc: The serum zinc concentration did not differ significantly between different groups. Similarly, Das *et al.* (2003) and Singh *et al.* (2005) reported non-significant

Table 1. Effect of different therapeutic protocols on Progesterone (ng/ml) and Estrogen (pg/ml) level during estrus induction in anestrus cattle (Mean±SE).

Parameter	Group	Days					
		0	7	14	21	42	60
Progesterone	A	0.06±0.01	0.09±0.02	0.11±0.02	0.14±0.04	0.77±0.50	1.04±0.76
	B	0.09±0.02	0.13±0.04	0.19±0.05	0.75±0.53	0.86±0.59	1.63±0.86
	C	0.14 ± 0.03^{a}	0.22 ± 0.06^{a}	0.82 ± 0.54^{ab}	0.97 ± 0.58^{ab}	1.65 ± 0.77^{ab}	2.98 ± 0.82^{b}
	D	0.13 ± 0.04^{a}	0.23 ± 0.07^{a}	0.75 ± 0.51^{ab}	1.50 ± 0.77^{ab}	2.17 ± 0.87^{ab}	3.06 ± 0.91^{b}
Estrogen	A	4.01±0.39	4.81 ± 0.41^{A}	5.72±0.80	7.61±3.18	8.04 ± 1.36^{A}	5.54 ± 0.35^{A}
	B	4.84±0.30	5.58 ± 0.31^{A}	8.79±2.60	5.40±0.47	7.68 ± 1.66^{A}	9.30 ± 1.00^{B}
	C	4.59±0.43	7.48 ± 1.33^{B}	7.92±2.42	9.25±2.68	8.62 ± 1.06^{A}	5.50 ± 0.24^{A}
	D	5.63±0.23	8.30 ± 1.60^{B}	6.21±0.53	8.73±2.35	11.17 ± 1.88^{B}	11.76 ± 1.41^{B}

Values with different superscripts differ significantly ($P \leq 0.05$) between days

Table 2. Effect of different therapeutic protocols on Mineral Profile during estrus induction in anestrus cattle (Mean±SE).

Mineral	Group	0 day	21st day	42nd day	60th day
Calcium (mg/dl)	A	7.75±0.10^{aA}	7.75±0.08^{aA}	7.81±0.06^{aA}	7.83±0.05^{aA}
	B	8.21±0.02^{aB}	8.34±0.03^{bC}	8.41±0.02^{bC}	8.66±0.03^{cC}
	C	7.90±0.03^{aA}	8.03±0.05^{abB}	8.16±0.04^{bB}	8.31±0.02^{cB}
	D	7.89±0.03^{aA}	8.25±0.03^{bC}	8.36±0.03^{cC}	8.75±0.02^{dC}
Phosphorus (mg/dl)	A	4.91±0.353	4.92±0.361	4.95±0.375	4.94±0.378
	B	5.24±0.405	5.26±0.405	5.29±0.405	5.32±0.401
	C	5.52±0.386	5.54±0.381	5.52±0.393	5.53±0.396
	D	6.19±0.023	6.21±0.021	6.24±0.02	6.27±0.02
Magnesium (mg/ml)	A	0.16±0.002^{aA}	0.16±0.003^{aA}	0.17±0.004^{aA}	0.17±0.004^{aA}
	B	0.17±0.002^{aB}	0.18±0.002^{abB}	0.18±0.002^{abB}	0.18±0.002^{bB}
	C	0.17±0.002^{aB}	0.17±0.003^{aAB}	0.18±0.003^{aAB}	0.18±0.003^{aAB}
	D	0.17±0.001^{aB}	0.18±0.001^{bB}	0.18±0.001^{abB}	0.19±0.001^{bB}
Copper (µg/ml)	A	0.66±0.007	0.66±0.007	0.66±0.007	0.66±0.008
	B	0.66±0.008	0.66±0.008	0.66±0.008	0.66±0.008
	C	0.67±0.010	0.67±0.010	0.67±0.010	0.67±0.010
	D	0.68±0.001	0.68±0.002	0.69±0.002	0.69±0.002
Cobalt (µg/ml)	A	0.50±0.016	0.50±0.017	0.50±0.0171	0.51±0.016
	B	0.51±0.017	0.51±0.017	0.52±0.016	0.52±0.016
	C	0.52±0.023	0.52±0.023	0.52±0.023	0.52±0.023
	D	0.56±0.001	0.56±0.001	0.56±0.001	0.56±0.001
Zinc (µg/ml)	A	1.14±0.014^{A}	1.14±0.014^{A}	1.16±0.017	1.16±0.018
	B	1.17±0.015^{AB}	1.17±0.015^{AB}	1.17±0.016	1.17±0.016
	C	1.18±0.016^{AB}	1.18±0.016^{AB}	1.18±0.016	1.18±0.016
	D	1.20±0.002^{B}	1.21±0.002^{B}	1.21±0.002	1.21±0.002
Iron (µmol/L)	A	11.51±0.49^{aA}	16.82±0.78^{abA}	21.45±1.31^{bcA}	26.22±1.76^{cA}
	B	11.61±0.81^{aAB}	15.73±0.10^{bB}	19.73±1.18^{cBC}	23.21±1.12^{Dbc}
	C	9.63±0.66^{aB}	11.81±0.67^{bB}	13.74±0.63^{cAB}	15.53±0.74^{dB}
	D	10.62±0.84^{aAB}	12.83±0.49^{bB}	14.25±0.68^{cC}	15.31±0.56^{dC}
Selenium (ppm)	A	0.07±0.011	0.07±0.012	0.08±0.012	0.08±0.008^{A}
	B	0.07±0.014	0.08±0.015	0.09±0.015	0.10±0.015^{AB}
	C	0.09±0.014	0.09±0.012	0.08±0.017	0.11±0.011^{AB}
	D	0.10±0.006^{a}	0.11±0.005^{ab}	0.12±0.005^{ab}	0.13±0.005^{bB}

Values with different superscripts (a, b, c, d) in a row and (A, B, C, D) in a column differ significantly (P ≤ 0.05) for same parameter.

difference between anestrus and cyclic cows.

Serum Iron: The plasma iron values were within normal physiological range but the values differed significantly (P≤0.05) within group and among groups. Conversely, higher and lower plasma iron level was reported by Dutta *et al.* (2001b) and Ramakrishna (1997) in anestrus cows.

Serum Selenium: The serum selenium level varies significantly at day 0 and day 60 in group D. Selenium deficiency affects cattle and sheep fertility (Hidiroglou, 1979), while high concentrations of selenium reduce the incidence of anestrus (Harrison *et al.*, 1984).

CONCLUSION

In conclusion, mineral mixture and mineral mixture plus double PG protocol improve the conception rate up to the extent of 50% and 83.33%, respectively. Therefore, mineral mixture supplementation with double PG protocol may be the best solution to combat postpartum true anestrus.

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