RESEARCH ARTICLE

Effect of Clomiphene Citrate, Co-synch Protocol and Mineral Mixture Supplementation on Estrous Induction and Hormonal Profile in Anestrus Sahiwal Cows

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Abstract

The present work was carried out on thirty Sahiwal cows selected from different villages in and around Rewa city of Madhya Pradesh. They were divided into 5 equal groups (n=6 each) to study the effect of clomiphene citrate, co-synch protocol and mineral supplementation alone or in combination on estrus induction, hormone concentration and fertility. Group 1, animals were fed ration containing 0% mineral mixture; Group 2, were fed ration containing clomiphene citrate (300 mg/ day for 5 days). In Group 3, the animals were subjected to co-synch protocol with fixed-time artificial insemination (FTAI) on day 11. Group 4 animals were fed ration containing a mineral mixture 1% and clomiphene citrate (300 mg/ day for 5 days). In Group 5, the animals were fed ration containing mineral mixture 1% and subjected to co-synch protocol. A significant difference was seen in progesterone concentration on day 42 and 60, while in estrogen, a significant difference was seen on day 9. Maximum conception rate (60%) was observed in animals that were supplemented with mineral mixture along with co-synch protocol (G5) followed by 50% with co-synch protocol alone (G3), and least conception rate (*i.e.,* 0%) was observed in control group (G1). In group 4, mineral mixture supplementation with clomiphene citrate, no significant increase in fertility rate (33.33%) was noted. However, a higher estrus induction rate (50%) was observed, whereas in group 5 mineral mixture supplementation along with co-synch protocol increased the estrus induction (83.33%) and fertility rate (upto 60%) in anestrus Sahiwal cows.

Keywords: Clomiphene citrate, Co-synch protocol, Estrus, Hormones, Sahiwal cows.

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Introduction

The dairy and livestock sector plays an important role in India's economy by contributing close to one-third of gross income of rural households and nearly half of the gross income in the case of those without land. Among the indigenous cow breeds, Sahiwal is known to be the best milch breed of India and has shown excellent adaptability to the agro-climatic condition of Madhya Pradesh state, with an average milk production potential of 2100 liter in 300 days of lactation length (Singh *et al.*, 2005). It has been noticed that the normal productive and reproductive behavior in domestic animals is closely associated with an interaction between hormonal and nutritional status of the animal (Kumar *et al*., 2013). In this regard, minerals, particularly some major and trace elements, play a crucial role. Mineral deficiency in animals may be related either to low intake or to the antagonistic effect of different minerals (Arthington and Ranches, 2021). The deficiency of single and combined minerals and their imbalances may cause anestrus and deprived conception (Keshari *et al*., 2021).

The efficacy of clomiphene citrate for improving fertility in anestrus condition with inactive and smooth ovaries have been reported (Kankal *et al*., 2008, Ingawale *et al*., 2011; More, 2013) in cows and buffaloes. Co-synch protocol allows the treatment of cows with sub-estrus or ovulation problems. Much work has been done using Co-synch methods on fertility response in normal cyclical and postpartum anestrus

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cows. However, reports on its application for fertility improvement in repeat breeding cows are meagre (Barolia *et al*., 2016). Keeping in view the major problem of anestrus in cows under field conditions and the role of Clomiphene citrate, Co-synch protocol and minerals in improving fertility, the present study was planned to evaluate fertility response and plasma profile of sex steroids following use of these approaches individually or in combination in anestrus Sahiwal cows under field condition.

Materials and Methods

The present investigation was carried out on animals presented at the Department of Veterinary Gynaecology and Obstetrics of the College of Veterinary Science & Animal Husbandry, Rewa (MP) and different villages in and around Rewa. Thirty anestrus cows were selected and randomly divided in five equal Groups each of 6 animals. Group-1 was controlling, where animals were fed ration as per farmers practice without any mineral supplement. Animals of Group-2 were treated with clomiphene citrate 300 mg for 5 days orally, Group-3 were subjected to co-synch protocol with FTAI on second GnRH, Group-4 were given the combination of clomiphene citrate along with 1.0% mineral mixture supplementation and Group-5 were subjected to co-synch protocol along with 1.0% mineral mixture.

The cows from all the five groups were observed for estrus signs in the morning and evening. The tonicity of the uterus was confirmed by per-rectal palpation and the time required for onset of estrus was recorded after last dose of clomiphene citrate (day 5) in Groups 2 and 4. Those cows that responded to the treatment were inseminated with frozen semen. Pregnancy diagnosis was performed on day 60 post-insemination by rectal palpation. The estrus induction response and first service conception rate were compared between groups.

Blood samples were collected by jugular venipuncture in heparinized vials, on day 0, 7, 14, 21, 42 and 60 of treatment for assay of plasma progesterone and estrogen concentration. The quantitative determination of progesterone concentration (ng/mL) was performed by ELISA using kits supplied by Cayman Chemicals, USA. The RIA technique estimated estrogen using diagnostic I^{125} kits supplied by Immunotech, France from BARC, Mumbai. The results of progesterone and estrogen concentration were correlated with the fertility response to treatment.

The data was analyzed using the standard statistical procedure as per Snedecor and Cochran (1994) using IBM ® SPSS software, version 22 statistical packages.

Results and Discussion

The mean concentrations of plasma estrogen and progesterone from day 0 to 60 in control Group 1 and treatment Groups 2, 3, 4 and 5 are presented in Tables 1 and 2, respectively. The plasma estrogen concentrations (pg/ml) varied significantly ($P \leq 0.05$) among the groups on day 9, being significantly higher in group 3 and group 5 compared to other groups. These results suggested that the maximum number of cows came into heat in the Co-synch treated group compared to the Clomiphene citrate treated group. These findings concurred well with those of Patterson *et al.* (2003), Bhowmik *et al.* (2014) and Dayanidhi *et al.* (2016). Estrogen is produced by the ovarian follicles, as the follicles grow,

Table 1: Effect of Clomiphene citrate, Co-synch protocol and mineral mixture supplementation on plasma estrogen concentrations (pg/mL) of Sahiwal cows

	Days of treatment/supplement						
Group	*0*	*7*	*9*	*14*	*21*	*42*	*60*
G1	4.12 ± 0.23	4.66 ± 0.38	4.7 ± 0.31^{a}	5.1 ± 0.51	4.79 ± 0.52	7.9 ± 2.38	5.47 ± 0.35
G2	4.84 ± 0.30	8.21 ± 2.72	8.46 ± 2.98^{ab}	5.97 ± 0.11	5.4 ± 0.47	5.18 ± 0.5	5.94 ± 0.4
G3	4.58 ± 0.21^{A}	5.2 ± 0.41^{A}	16.45 ± 3.35^{Bbc}	5.5 ± 0.29^{A}	6.43 ± 0.42^{A}	5.69 ± 0.32^{A}	5.5 ± 0.24^{A}
G4	4.61 ± 0.14	10.67 ± 3.14	8.77 ± 2.35^{ab}	6.2 ± 0.53	6.24 ± 0.30	6.42 ± 0.15	6.32 ± 0.19
G5	4.67 ± 0.14^{A}	5.7 ± 0.41^{A}	19.11 ± 2.62^{Bc}	6.2 ± 0.53^{A}	6.35 ± 0.27^{A}	6.56 ± 0.02^{A}	6.15 ± 0.22^{A}

Values with different superscripts in capital letters (A, B, C, D) in a row and small letters (a, b, c, d) in a column differ significantly ($P \leq 0.05$).

Table 2: Effect of Clomiphene citrate, Co-synch protocol and mineral mixture supplementation on plasma progesterone concentration (ng/ml) in Sahiwal cows

	Days of treatment/supplement						
Group	*0*	*7*	*9*	*14*	*21*	*42*	*60*
G1	3.73 ± 0.23	4.23 ± 0.31^{b}	4.31 ± 0.28^{bc}	4.28 ± 0.23	4.3 ± 0.18	4.27 ± 0.26	4.56 ± 0.31
G2	4.16 ± 0.30	3.25 ± 0.73^{ab}	3.1 ± 0.72^{b}	4.22 ± 0.20	4.12 ± 0.50	4.66 ± 0.66	4.49 ± 0.79
G3	3.92 ± 0.21^{AB}	2.71 ± 0.50^{ABab}	1.76 ± 0.89^{Aab}	4.11 ± 0.16^{AB}	4.57 ± 0.33^{AB}	5.05 ± 0.83^{AB}	5.49 ± 1.06^{B}
G4	3.93 ± 0.14^{AB}	2.21 ± 0.67^{abA}	2.66 ± 0.64^{abAB}	4.21 ± 0.21^{AB}	4.67 ± 0.42^{AB}	4.77 ± 0.62^{AB}	4.85 ± 0.94^{B}
G5	3.97 ± 0.14^{B}	1.54 ± 0.06^{Aa}	0.75 ± 0.01^{Aa}	4.46 ± 0.11^{B}	5.39 ± 0.40^{BC}	5.85 ± 0.73^{BC}	6.83 ± 1.04^{C}

Values with different superscripts in capital letters (A, B, C, D) in a row and small letters (a, b, c, d) in a column differ significantly ($p \leq 0.05$).

more estrogen is produced. It acts in a positive feedback mechanism and is responsible for LH surge and ovulation. It also affects cow's nervous system, causing restlessness, phonation, mounting, and most importantly, the willingness to be mounted by other animals. Its deficiency and irregular production is seen in anestrus animals.

The plasma progesterone levels varied significantly ($p \leq 0.05$) between days among groups 3, 4 and 5. The plasma progesterone levels also varied significantly ($p \leq 0.05$) between groups on day 7 and 9 of treatment, mainly due to injection prostaglandin and GnRH used on these days under Co-synch protocol in G3 and G5 (Table 2). In groups 3 and 4, the plasma progesterone levels were lowest on day 0 and 7, respectively, and highest on day 60 ($P \leq 0.05$) with statistically similar values on all other days, while in Group-5 it was significantly ($P \leq 0.05$) lower on day 7 and 9, and higher on day 60, with statistically similar values on all other days. On day 60, Group-5 had slightly more mean progesterone level compared to Groups-3 and 4, because of pregnancy in more cows. On day 9, Group-5 as compared to Group-1 and 2 had lower progesterone levels because more cows came in estrus in this group, and Group 3 and 4 were statistically similar and intermediate (Table 2)..

Serum progesterone concentration is specifically related to the cow's CL function and fertility. The progesterone concentration is responsible for stimulating cyclicity, follicular development, and continuation of pregnancy. In normal cyclic animals, serum progesterone level is expected to be high during diestrus stage and subsequently should reduce during estrus. The findings of serum progesterone concentrations (ng/mL) were similar to those of Ayres *et al.* (2013) in dairy cows. Serum progesterone concentrations of Group 4 and 5, supplemented with the mineral mixture and Clomiphene citrate and Co-synch protocol were significantly higher and in close agreement with Ojha *et al.* (2018). The trend observed in the present study indicates the improvement in estrus and conception rate, the profile of progesterone increased after supplementation compared to that without supplement.

Estrus Response and Conception

The onset of estrus was detected by cervico-vaginal discharge and per-rectal examination. Out of 6 cows in each group, cows exhibited estrus under groups G1, G2, G3, G4 and G5 were 1, 2, 4, 3 and 5, respectively. Further, the numbers of cows conceived in respective groups were 0, 1, 2, 1 and 3. The results in terms of estrus response and conception rate were better in co-synch with mineral supplement followed by co-synch group alone than others. Conception rates in groups G1, G2, G3, G4 and G5 were 0.00, 50.00, 50.00, 33.33 and 60.00%, respectively, among the responded cows. Conception rate was higher in Group-5 followed by Groups-2 and 3 as compared to others. Among the overall treated cows, highest conception was recorded in G5 (50.00, 3/6) followed by G2 (33.33, 2/6) and G3 and G4 46.66, 1/6 each), while none conceived in control G1 group. The present findings in Clomiphene citrate treated group were lower than the conception rate (71.42%) recorded by Purohit and Bishnoi (1993) in anestrus Rathi heifers, while More (2013) reported 60% conception rate in crossbred cows. The conception rate reported by different researchers with Co-synch protocol varied from 22.70 to 66.66% (Geary *et al.,* 1998; Melendez *et al*., 2006; Amle *et al*., 2015; Barolia *et al*., 2016; Rajkumar *et al.,* 2021). The variation in pregnancy rate might be due to age, nutritional status, method of heat detection, type of breeding, a technique used for heat detection, season etc. In the present study, in Co-synch protocol the conception rate was comparable with earlier 66.66% reported by Barolia *et al.* (2016.

Conclusion

Co-synch protocol with mineral mixture supplementation gave a higher conception rate (60%) than clomiphene citrate (50%) alone or in combination with the mineral mixture in anestrus cows. Plasma progesterone and estrogen concentrations were diagnostic tools for identifying ovarian function.

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ANNOUNCEMENT: SVSBT-NS-2022

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The ***IX Annual Convention*** and ***National Seminar*** of The Society for Veterinary Science & Biotechnology ***(SVSBT) on "Recent Biotechnological Advances in Health and Management to Augment Productivity of Livestock and Poultry"*** will be **organized at Ramayanpatti, Tirunelveli - 627 358, Tamil Nadu, during September 22-24, 2022** (Thursday, Friday & Saturday) by Veterinary College & Research Institute, Tirunelveli - 627 358, TANUVAS, (TN). The detailed Brochure cum Invitation showing Theme Areas/ Sessions, Registration Fee, Bank Details for online payment and deadlines, etc. has been floated on the Whats Apps and e-mails. Accordingly, the organizing committee of ***SVSBT NS-2022 invites abstracts*** of original and quality research work on theme areas of seminar limited to 250 words by e-mail on svsbttnns2022@gmail.com or mopandian69@gmail.com latest by 30th August, 2022 for inclusion in the Souvenir cum Compendium to be published on the occasion.

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