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Efficiency of Copper and Selenium during Estrus Synchronization on Estrus Behaviour, Estradiol and Progesterone Concentration in Salem Black Goats

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ABSTRACT

Background: Copper and selenium were proven modulaters of reproductive function in ruminants under dietary supplementation. Their effects under pareneral administraton during estrus synchronization are not clearly defined. On this background the present was aimed to evaluate the effect of copper and selenium on estrus associated responses in goats.

Methods: Thirty adult female Salem black goats were selected. On day ten of the estrus cycle, 250 µg of cloprostenol injection was given intramuscularly to all the animals and assigned to five groups. GI: control, GII: 4 µg of Buserelin, GIII: 20 mg cupric chloride, GIV: 10 mg sodium selenite and GV: copper plus selenium injections were administrated. Whole blood was collected for seven days for hormone assay. Estrus was detected using aproned bucks.

Result: There was no significant (p>0.05) difference in estrus response, onset and duration of estrus between groups. The progesterone concentration decreased significantly (p<0.05) from day 0 (before $PGF_2\alpha$) to day 1 (after $PGF_2\alpha$) in all groups. There was a significant (p<0.05) increase in estradiol and progesterone concentration in the GnRH group compared to other groups. Parenteral administration of copper and selenium did not significantly improve estrus behavior, estradiol and progesterone concentration in Salem black goats. GnRH was once again proved to be effective in the improvement of estrus associated responses in goats.

Key words: Copper, Estrus, Goats, GnRH, Hormones, Selenium.

INTRODUCTION

The goat is considered as “poor man’s cow” and provides livelihood for billions of low and medium input farmers (Gupta *et al.,* 2019). As India is an agricultural based country, goats are predominantly distributed in India with a population of 148.88 million which constitutes to 27.80% of the total livestock population (Basic Animal Husbandry Statistics, 2019). The increased demand for goat production is attributed to their regular supply of milk, meat and cashmere. Although, estrus synchronization in goats with progesterone (Pietroski *et al.,* 2013, Hashemi and Safdarian, 2018), estradiol benzoate (Souza-Fabjan *et al.,* 2017), double $PGF_2\alpha$ injections and Ovsynch protocols (Riaz *et al.,* 2012) have been adopted to improve goat population, the estrus response was not always 100% and the conception rates were below 70% (Menchaca *et al.,* 2007; Riaz *et al.,* 2012). There is further necessary to tighten the existing synchronization protocols with novel substances that could modulate ovarian function. This research gap is of a greater challenge to the scientific community in the increasing population scenario to enhance the livestock production.

Trace minerals such as copper (Garcia-Diaz *et al.,* 2012; Roychoudhury *et al.,* 2014; Soni *et al.,* 2018) and selenium (Basini and Tamanani *et al.,* 2000; Gabryszuk *et al.,* 2002; Koyuncu and Yerlikaya 2007; Yao *et al.,* 2018) were largely found to regulate ovarian granulosa cell estradiol synthesis and estrus behaviour in ruminants. Copper supplementation *in vivo* significantly increased estradiol, progesterone, FSH and LH concentration in Simmental heifers (Wang *et al.,* 2008). Similarly, selenium supplementation *in vivo* significantly increased follicular growth (Vázquez-Hernández *et al.,* 2017), estrus response (Gabryszuk *et al.,* 2002; Koyuncu and Yerlikaya 2007; Wani *et al.,* 2016) and conception rate (Ganie *et al.,* 2014; Musa *et al.,* 2018) in ruminants. The existing evidences are based on prolonged dietary supplementation and/or multiple parenteral administrations of copper and selenium, while studies are meager on single parenteral administration of copper and selenium in combination with $PGF_2\alpha$ during estrus synchronization protocols. In view of these considerations an attempt has been made to evaluate the effect of parenteral administration of copper and selenium

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in comparision to GnRH on estrus behaviour and changes in estradiol and progesterone hormone concentration in Salem black goats.

MATERIALS AND METHODS

Animal management

The present study was conducted in the year 2017-2018 in an experimental livestock unit, ICAR-National Institue of Animal Nutrition and Physiology (NIANP), Adugodi, Bengaluru, Karnataka, India. Thirty adult female Salem black goats of twelve to eighteen months of age were selected. The animals were housed in a well-ventilated shed and maintained under proper hygienic conditions. The animals were fed as per ICAR feeding standards (Ranjhan, 1998).

Experimental design

Prior to the start of the experiment, all the animals were injected with cloprostenol 250 µg/animal (Pregova, Virbac) to lyse the corpus luteum present if any. Ten days (day 0 of the experiment) after the first injection, the second injection of cloprostenol was injected and the animals were alloted to the following groups. Group I (GI, n=6) served as the control, Group II (GII, n=6) animals were given intramuscular injection of 4 µg of Buserelin, GnRH analog (Pregulate, Virbac). Group III (GIII, n=6) animals were given intramuscular injection of cupric chloride (Himedia) @ 20 mg per animal. Animals in Group IV (GIV, n=6) were given intramuscular injection of sodium selenite (Sigma Aldrich) @ 10 mg per animal. Groups V (GV, n=6) animals were given intramuscular injections of cupric chloride @ 20 mg plus sodium selenite @ 10 mg per animal. The experiment was replicated thrice with a gap of 60 days and the same grouping was followed for each experiment.

Estrus detection

Estrus was determined initially by signs such as frequent tail wagging, bleating, swollen vulva and vulval mucous discharge. Further confirmation was made by exposing the does to apronised bucks. The female does which stand still on being mounted were considered to be in estrus. Estrus percent was calculated by total number of animals responded in a group divided by total animals of animals in a group. Since the experiment was replicated thrice the percent estrus response of three experiments were used for statistical analysis. The onset and duration of estrus was also recorded.

Blood collection and estimation of plasma estradiol and progesterone concentration

Whole blood was collected from day 0 to day 7 from all the animals. The plasma was separated by centrifugation at 3500 rpm for 15 min at room temperature and stored at -20°C for estimation of estradiol and progesterone hormone concentration. Plasma estradiol (Tsang *et al.,* 1980) and progesterone (Radwanska, 1978) concentration was determined by enzyme linked immunosorbent assay (ELISA) using a microplate reader (Thermo Scientific) by ELISA kits (Cal biotech kits).

Statistical analysis

The data obtained on various parameters were statistically analyzed using Graphpad PRISM software 6.0. The differences between means of different groups were analyzed by one way ANOVA and multiple comparisons were performed by Tukey'stest. The percentage values of estrus response were converted to arcsine values before statistical analysis and significance was determined at $P<0.05$.

RESULTS AND DISCUSSION

Trace minerals hold critical roles in maximizing reproductive efficiencies in ruminants. The trace elements likely to be of most practical significance are copper, selenium and zinc (Pal, 2015). It has been established that copper has a stimulatory effect on the release of GnRH and FSH (Rouchoudhary *et al.,* 2016) which triggers the synthesis and release of estradiol. Furthermore, copper inpart may promote ovarian granulosa cell estradiol synthesis via mRNA expression of *Cytochrome P450 Family 19 subfamily A member 1(CYP19A1)* which encodes the enzyme aromatase that converts androgens to estradiol in the ovarian granulosa cells (Soni *et al.,* 2018; Tej *et al.,* 2021). Selenium was found to stimulate ovarian granulosa cell estradiol synthesis via increase in the number of follicles (Vázquez-Hernández *et al.,* 2017) granulosa cell proliferation (Basini and Tamanini, 2000), increased activity of antioxidant enzymes, reduce lipid peroxidation (Said *et al.,* 2012) and upregulation of genes involved in estradiol synthesis signalling pathways (Yao *et al.,* 2018). These evidences suggest that, trace minerals such as copper and selenium could be possibly used to improve estrus response in synchronization protocols.

Effect of copper and selenium on estradiol concentration

Within-group between days comparison (Fig 1) revealed no significant ($p>0.05$) increase in estradiol concentration from day 0 to day 7 in all the groups. Between group comparison (Fig 2) revealed significantly higher ($p<0.05$) estradiol concentration in GnRH group compared to other groups, while the estradiol concentration did not differ significantly ($p>0.05$) between control, copper, selenium, copper plus selenium groups. The significant ($p<0.05$) increase in estradiol concentration in GnRH group compared to other groups could be attributed to GnRH injection which stimulates FSH secretion from the anterior pituitary and subsequent follicular growth and estradiol synthesis from granulosa cells (Marques *et al.,* 2018). No significant ($p>0.05$) effect of copper and selenium on estradiol concentration was observed in the present study. Contradictory to the present findings, dietary supplementation of copper sulphate (Wang *et al.,* 2008) significantly increased estradiol concentration in ruminants. Moreover, oral supplementation of sodium selenite in ewe (Sen *et al.,* 2011) and selenium rich yeast in Taihang black goats (Shi *et al.,* 2018) significantly increased estradiol concentration. The significant increase in estradiol concentration with copper and selenium in other studies could be due to

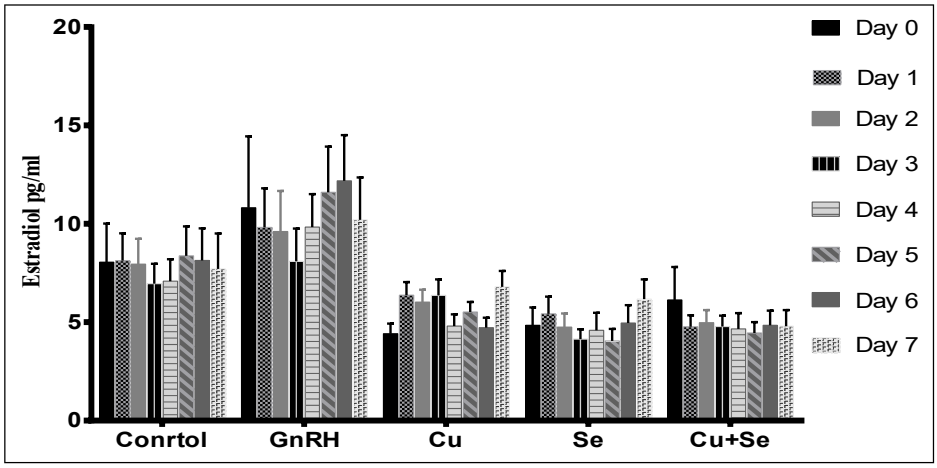

Fig 1: Changes in estradiol concentration (pg/ml) from day 0 (before $PGF_2\alpha$) to day 7 in different groups of Salem black goats. The graph represents within group between days comparison of estradiol concentration from day 0 to day 7, p<0.05 (n=18).

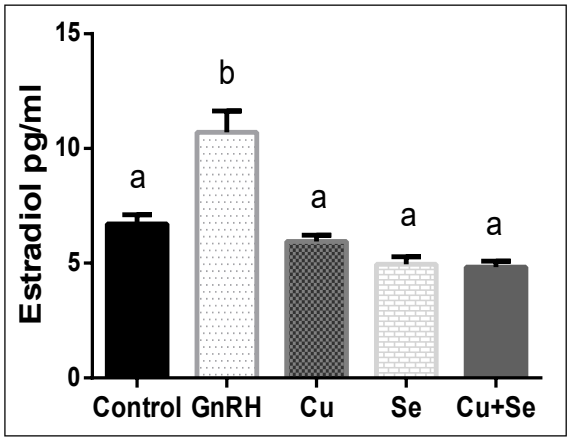

Fig 2: Overall estradiol concentration (pg/ml) in different groups of Salem black groups. The graph represents the average of estradiol concentration from day 0 to day 7, bars bearing different superscript (a,b) differs significantly, p<0.05 (n=18).

prolonged dietary supplementation. The estradiol concentration observed in the present study was in agreement with other reports where a similar estradiol concentration of 7.7±1.7 pg/ml in Dwarf goats (Khanum *et al.,* 2008) and 5.19±0.71 pg/ml in Huanghuai Goats (Pang *et al.,* 2009) was observed.

Effect of copper and selenium on progesterone concentration

Within group between days comparison (Fig 3) revealed that the progesterone concentration was significantly higher (p<0.05) on day 0 (before $PGF_2\alpha$ injection) in all the groups and the concentration decreased significantly (p<0.05) on day 1 and continued to maintain at significantly (p<0.05) lower levels till day 5 in all the groups. Between groups comparison (Fig 4) revealed significantly (p<0.05) higher progesterone concentration in GnRH group compared to other groups, while the progesterone concentration did not differ significantly (p>0.05) between control, copper, selenium, copper plus selenium groups. A significant (p<0.05) decline in progesterone concentration from day 0 to day 1 could be due to $PGF_2\alpha$ injection which selectively lyses the developing corpus luteum (CL) (Omontese *et al.,* 2016) and subsequent decrease in progesterone concentration. A significant (p<0.05) increase in progesterone concentration in the GnRH group compared to other groups could be attributed to GnRH injection which would have stimulated LH secretion and subsequent progesterone synthesis from luteal cells (Marques *et al.,* 2018). The study revealed no significant (p>0.05) effect of copper and selenium on progesterone concentration in Salem black goats. Contradictory to our findings dietary supplementation of copper sulphate (Wang *et al.,* 2008) significantly increased progesterone concentration in ruminants. Moreover, oral supplementation of sodium selenite (Kamada *et al.,* 2014), selenium (Cerny *et al.,* 2016) selenium enriched yeast (Shi *et al.,* 2018) significantly increased progesterone concentration in ruminants which may be due to prolonged dietary supplementation of copper and selenium. The progesterone concentration observed in the present study was in agreement with other reports where a progesterone concentration of 0.1 to 5.4 ng/ml in Dwarf goats (Khanum *et al.,* 2006), 0.1±0.03 to 7.7±0.6 ng/ml in Dwarf goats (Khanum *et al.,* 2008) and 0.51±0.10 to 10.72±0.71 ng/ml in Markhoz Goat (Farshad *et al.,* 2008) was observed respectively.

Effect of copper and selenium on estrus response, onset and duration of estrus

The mean±SEM values of onset, duration and estrus response were presented in Table 1. There was no significant (p>0.05) difference in onset, duration and estrus response between groups. In the estrus cycle of the goat, at the end of diestrus phase $PGF_2\alpha$ secreted from the uterine

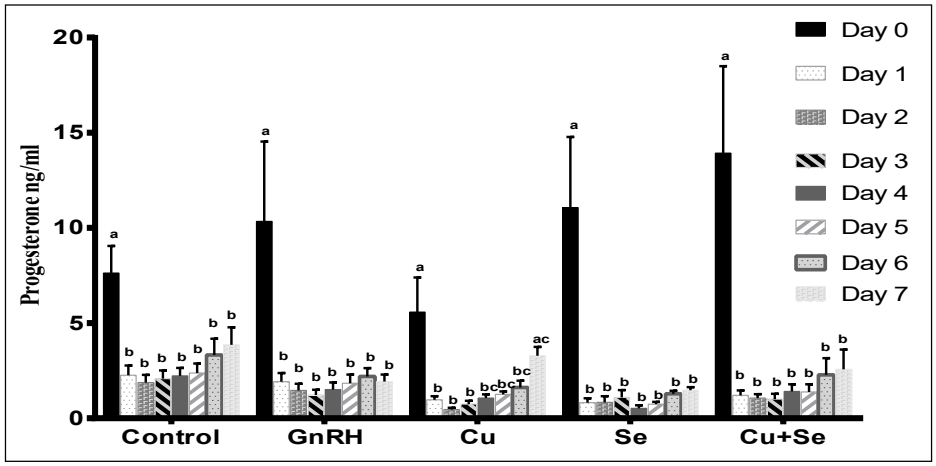

Fig 3: Changes in progesterone concentration (ng/ml) from day 0 (before PGF2α) to day 7 in different groups of Salem black goats. The graph represents within group between days comparison of progesterone concentration from day 0 to day 7. Bars bearing different superscript (a,b) with in a group differs significantly, p<0.05, (n=18).

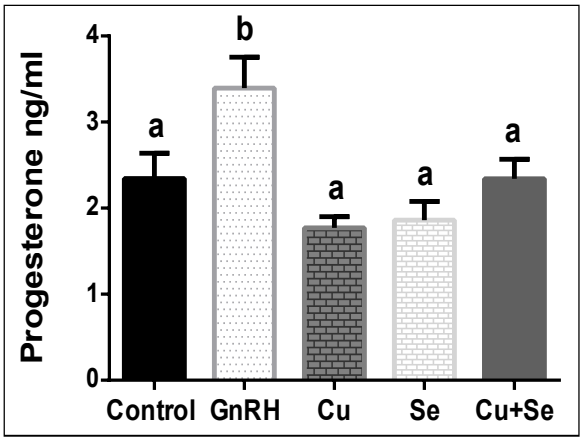

Fig 4: Overall progesterone concentration (pg/ml) in different groups of Salem black goats. The graph represents the average of progesterone concentration from day 0 to day 7, bars bearing different superscript (a,b) differs significantly, p<0.05 (n=18).

endoemetrium lyse the CL, lowering the progesterone concentration. This reduced progesterone triggers the secretion of GnRH from hypothalamus and subsequent secretion of FSH from anterior pituitary. Under the influence of tonic FSH secretions the existing dominant follicle of the follicular wave continuous to secrete estradiol which induce estrus behaviour and when the estradiol concentration reaches peack it triggers ovulation (Fatet *et al.,* 2011). In the current study, low estrus response was noticed in control groups ($PGF_2\alpha$ alone). Similar to our findings, a low estrus response of 4.3-15.8% in Red Sokoto goats (Tauheed *et al.,* 2010), 30% in Persian downy does (Hashemi and Safdarian, 2018) and 0% in Salem black goats (Gupta *et al.,* 2019) was observed in goats synchronized with double $PGF_2\alpha$ protocol. Relatively, a high estrus response of 50% was observed in GnRH group. Similar estrus response was observed in our previous study on Salem black goats where GnRH based Ovisynch Protocol was used (Gupta *et al.,* 2019). This effect may be attributed to GnRH injection which would have directly trigerred hypothalamic-pituitary-ovarian axis. Furthermore, low estrus response was observed in copper and selenium groups. No similar studies were conducted so far to compare our results but selenium when injected with sponge impregnated with progesterone it resulted in 90% (Qureshi *et al.,* 2010), 88% (Wani *et al.,* 2016) and 100% (Mujawar *et al.,* 2019) estrus response in buffaloes.

The onset of estrus observed in the present study fall in line with other studies where double $PGF_2\alpha$ injection resulted in onset of estrus of 45-45.5 h in Black bengal does (Khandoker *et al.,* 2009) and 47.7±10.1 h in Alpine, Saanen and Toggenburg cross (Esteves *et al.,* 2013). The finding of the present study was in agreement with other reports where goats synchronized with $PGF_2\alpha$ showed a duration of estrus of 25-51h in Black bengal does (Khandoker *et al.,* 2009) and 26.8±15.0 h in Alpine, Saanen and Toggenburg cross (Esteves *et al.,* 2013) respectively. The onset and duration of estrus observed in the present study falls within the range as that of other breeds of goats and this information is important for the determination of the time of mating and or insemination to maximize reproductive efficiency in goats.

In the present study, copper and selenium did not significantly affect estradiol, progesterone, estrus response, onset and duration of estrus, while GnRH injection significantly improved estrus associated responses compared to other groups. Whereas, from other studies it was evident that selenium and progesterone combination resulted in high estrus response in buffaloes (Qureshi *et al.,* 2010; Wani *et al.,* 2016; Mujawar *et al.,* 2019). The findings are suggestive of use of copper and selenium in combination with GnRH/progesterone devices to evaluate the effect on estrus associated responses. Moreover single injection of minerals just before estrus might have not improved estradiol synthesis significantly which may be due to the limitations imposed by other competing factor (Landau,

Table 1: Mean±SE values of onset, duration and estrus response in Salem black goats (n=18).

Parameters	Control	GnRH	Cu	Se	Cu+Se
Onset (h)	51.5±36.5	66.81±15.69	30.13±6.07	40.17±13.86	43.75±4.25
Duration (h)	39.75±8.75	37.97±5.15	39.81±4.80	24.00±4.36	47.75±8.25
Estrus response (%)	20.60±10.60	49.22±7.30	23.49±11.75	23.01±13	11.75±11.75

2000) as copper and selenium are involved in diverse functions, the given dose would have utilized for general body functions. While the significant effect of copper and selenium in other studies could be due to prolonged dietary supplementation and or multiple injections.

CONCLUSION

Although copper and selenium were proved to be estrogenic in ruminants, in the current study no significant effect was noticed. It was concluded that single parenteral injection of copper and selenium along with $PGF_2\alpha$ did not improve estrus behavior, estradiol and progesterone concentration in Salem black goats. Whereas GnRH was proved to be effective in improving the reproductive efficiency in goats. Therefore, the current study strongly hints a necessity for the use of combintion of GnRH/progesterone along with trace minerals. However, in the present study no such combinations were used. Further studies are focussed on use of copper and selenium in combination with GnRH/ progesterone devices.

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Compliance with ethical standards

The animal studies have been approved by the appropriate ethics committee, F.No25.08.2016- CPCSEA, (part-I), dt 20.05.2016, Govt. of India and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Conflict of interest

The authors declare that they have no competing interests.

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