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Effect of Nano Zinc Supplementation on Reproductive Performance of Assam Hill Goat

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ABSTRACT

Background: Interaction between nutrition and reproduction has been established to play an important role in reproductive performance of animals. Zinc is considered to be an essential element required for reproduction. The present study was conducted to evaluate the efficiency of nano zinc (NZn) as feed supplementation on reproductive performance of Assam Hill goats.

Methods: A total of twenty-four numbers of 7 days post kidding doe maintained at Goat Research Station, Assam Agricultural University, Burnihat were randomly selected and divided into 4 groups comprising 6 animals in each group. The control group animals were fed with basal diet without zinc supplementation however, all the animals of the treatment groups received 25 mg NZn, 35 mg NZn and 50 mg NZn/kg concentrate mixture with basal diet for a period of three months. Does were bred naturally at 24 hours from the onset of oestrus and confirmed for pregnancy after two months. Parturition behaviour was observed closely from two days before the expected date of kidding upto the period of placental expulsion.

Result: Supplementation of 50 mg NZn to the basal diet of postpartum Assam Hill Goat significantly improved the reproductive performance by decreasing postpartum oestrus interval and increasing conception rate (83.33%).

Key words: Assam hill goat, Conception rate, Nano zinc, Postpartum oestrus, Ultrasound.

INTRODUCTION

The 'poor man's cow'- goat is a popular animal among the rural and marginal farmers due to their ability to thrive under unfavorable climatic and managemental condition. Assam Hill Goat, the 31st registered goat breed in India (National Bureau of Animal Genetics Resources, NBAGR, Karnal, ICAR) are widely distributed throughout Assam and in some adjoining areas of Meghalaya, from the North Eastern region of India. They are well known for quality meat, high adaptability to local high humid agro climatic condition with high prolificacy (Zaman *et al*., 2013). Goats exhibit some special reproductive behaviour which is affected by various environmental and climatic factors. An improved nutrition enhances sexual behaviour in goats while under-nutrition and particularly negative energy balance adversely affect this activity. Improper nutritional management results in delayed puberty, ovulatory disturbances and lower conception rates, embryonic and fetal death, prolonged postpartum anoestrus period, poor lactation and improper neonatal growth and mortality in goat. Balanced nutrition by incorporation of various essential and trace minerals are utmost important for maintenance of good reproductive health of goat.

Zinc (Zn) as a trace mineral has an influencing role in reproduction by regulating certain reproductive hormone as well as growth and production. It is essential for proper sexual maturity, reproductive capacity and more specifically onset of estrus. Unlike other trace minerals, zinc cannot be stored in the body; therefore needs continuous supplementation to maintain optimum physiological functions in animals (Mandal *et al.,* 2008). Absorption of zinc in the body is very low and differs with the age of animal and the sites in the gastrointestinal tract. Among all the probable approach, recently the use of nanotechnology to produce nano size zinc called nano zinc (N-Zn) with sizes of 1 to 100 nm has received considerable attention due to their novel physiochemical properties resulting in greater specific surface area, higher surface activity, high catalytic efficiency, better bioavailability, smaller dose rate and stable interaction within the biological system (Swain *et al.,* 2016). However, very limited studies on the effect of supplementation of nano zinc in Assam Hill Goat with respect to the enhancement of reproductive efficiency is available in the literature. Therefore, the present study was aimed to determine the effect of dietary nano zinc supplementation on reproductive performance of Assam Hill Goat.

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MATERIALS AND METHODS

Selection of animals

A total of 24 of 7 days post kidding healthy does of Assam Hill Goat maintained at Goat Research Station, Assam Agricultural University, Burnihat, Assam, India were selected for the present study. The study was conducted for a period of one year commencing from November, 2021 to October, 2022. All the does were in 2nd to 3rd parity, body weight ranging from 15-20 kg and the animals were maintained under semi-intensive system of management. Goats were let loose for grazing during day time and were supplied with 200 g of concentrate mixture along with 3-4 kg chaffed fodder daily to each animal and water supply was *ad libitum*. All the does were vaccinated against peste des petits ruminants (PPR), enterotoxaemia and Foot and mouth disease (FMD). Deworming was done at 6-month interval in all the animals.

Feeding trial

Feeding trial was conducted with the following concentration of nano zinc (Swain *et al.,* 2019) to study the effect of nano zinc on reproductive performance. For this experiment a total of 24 numbers of 7 days post kidding does were randomly divided into four groups comprising six animals in each group.

T0 (Control): Does were fed the basal diet which consists of concentrate mixture.

T1 (Treatment): Does were fed the basal diet supplemented with 25 mg nano zinc/kg concentrate mixture/head/day for a period of 3 months.

T2 (Treatment): Does were fed the basal diet supplemented with 35 mg nano zinc/kg concentrate mixture/head/day for a period of 3 months.

T3 (Treatment): Does were fed the basal diet supplemented with 50 mg nano zinc/kg concentrate mixture/head/day for a period of 3 months.

The doses of nano zinc in T1, T2 and T3 were supplemented to the diets by dissolving the nano zinc in Phosphate buffer solution @ 1 mg/ml and does were fed with the help of a syringe.

Assessment of reproductive performance

All the experimental animals including animals of the control group were closely observed for the occurrence of oestrus with the help of a vasectomized buck and strict visual observation for external signs of oestrus. Oestrus does were bred naturally at 24 hours from the onset of oestrus. Reproductive traits like interval between kidding and occurrence of first postpartum oestrus, duration and intensity of oestrus, behavioural and physical signs of oestrus were recorded. Interval between kidding and occurrence of first postpartum oestrus was recorded as the time interval from the date of kidding till the date of exhibition of first observed oestrus after the corresponding kidding. Oestrus was detected using a vasectomized teaser buck and on the basis of behavioural and physical signs *viz.*, mucus discharge from vulva, wagging of tail, frequent urination *etc*. Duration of oestrus was considered as the time interval in hours between the onset and end of oestrus. The end of the oestrus was taken as mid-way between the last positive and subsequent negative acceptance of the teaser buck. Based upon the degree of manifestation of behavioural signs, oestrus was categorized to denote three different types of intensity *viz.*, intense, intermediate and weak. The does exhibiting particularly the behavioural signs of continuous bleating, restlessness, mounting on flockmates and loss of appetite were categorized that had shown intense oestrus. The does showing the signs of seeking male, sniffing of male and intermittent bleating were placed under intermediate oestrus and those revealing seldom intermittent bleating, sniffing of male and seeking male were considered to have weak oestrus. Behavioural signs of oestrus such as bleating was recorded as continuous and intermittent, wagging of tail as the rhythmical movement of tail from side to side or up and down was recorded as wagging of tail. Seeking male was recorded as when the doe moved to the buck and showed interest to it. Other behavioural signs recorded were mounting on flockmates, sniffing of male, standing to be mounted by teaser buck, restlessness and loss of appetite. Physical signs of oestrus such as quantity of vaginal mucus was recorded as scanty, when little amount of mucus was seen at the vulva and absent, when no mucus was observed. Colour of the vaginal mucus was recorded as clear when mucus was transparent and opaque when it was not clear. Consistency of vaginal mucus was considered as thin and thick. When a little amount of mucus taken between the tip of thumb and index finger forming a string or rope on drawing apart, it was considered as thick, and when no such string was formed, it was considered as thin. For spinnbarkeit a drop of mucus was taken in between the thumb and the forefinger and elasticity and stringiness of the mucus was recorded and graded by separating the thumb and forefinger as high, medium and low spinnbarkeit (Fig 1). Arborization

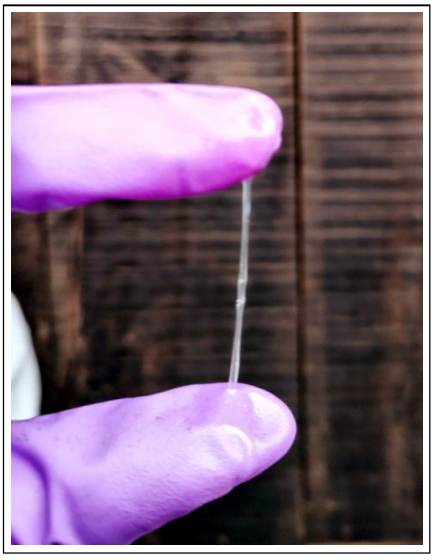


Fig 1: Spinnbarkeit test.

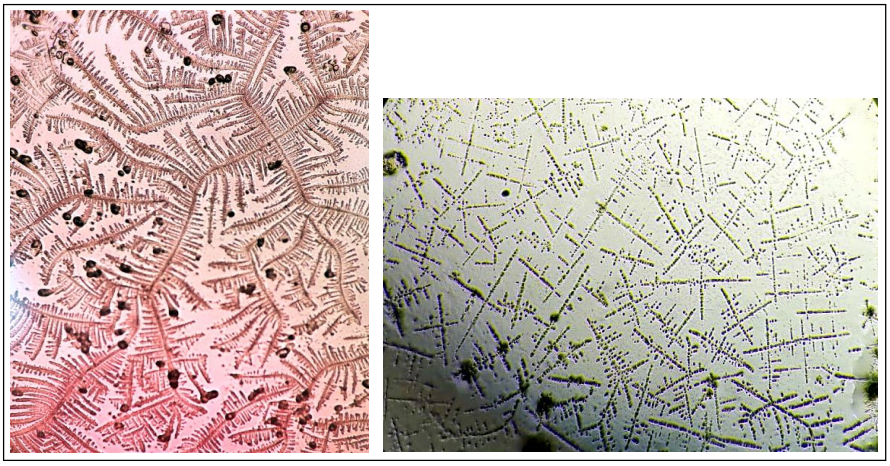


Fig 2: Typical and atypical fern pattern of cervico vaginal mucus at oestrus.



Fig 3: USG image showing cotyledon and foetus of pregnant doe.



Fig 4: USG image showing foetus and foetal fluid of pregnant doe.

was done as per the method of Bishnoi *et al.* (1982) where mucus was collected and spread uniformly over a clean dry glass slide and allowed the smears to dry completely in the air and was observed under a microscope (40X) for the appearance of crystallization pattern called fern pattern (Fig 2). The pattern was graded using classification of cattle that ranged from 0 to 4 on a scale.

Pregnancy in does was confirmed by Ultrasound Pregnancy Detector using the transabdominal and transrectal probe at two months after service (Fig 3, 4). Ultrasound scanning was done by using Fujifilm Sonosite M-Turbo™ portable Ultrasound Machine, Sonosite Inc. (USA) equipped with Sonosite L52X rectal and transabdominal Ultrasound probe.

Parturition

Duration of different stages and time required for the whole process of parturition along with placental weight and numbers of cotyledons were recorded. The pregnant does were separated from the flock 7 days before the expected date of parturition. The course of parturition was divided into three stages as per the description of Hafez (1985) for recording the signs of parturition (Fig 5). Each animal was observed closely from two days before the expected date of kidding upto the period of placental expulsion. The stages of parturition were recorded as first stage from the onset of restlessness to appearance of water bag at vulva, second stage from appearance of foetal hooves at vulva to appearance of foetal shoulders and finally the expulsion of whole foetus and third stage from expulsion of foetus till expulsion of foetal membrane. Total time period for completion of whole parturition process was calculated by adding the time required for first, second and third stages of parturition.

For placental weight the foetal membrane after expulsion were collected and cleaned immediately. Then the foetal membranes were placed into a pre weight paper and weight of the placenta was taken in a balance and expressed in gram (g). The foetal membranes were then cut open and spread on a paper and numbers of cotyledons were calculated.

Statistical analysis

Interval between kidding and occurrence of first postpartum oestrus, duration of oestrus, time required for different stages of parturition, weight of placenta and numbers of cotyledons were recorded as Mean±SE (Standard Error). Intensity of oestrus, behavioural and physical signs of oestrus and conception rate was expressed in percentage. Means were analyzed using one way analysis of variance followed by post hoc test to determine the significance difference

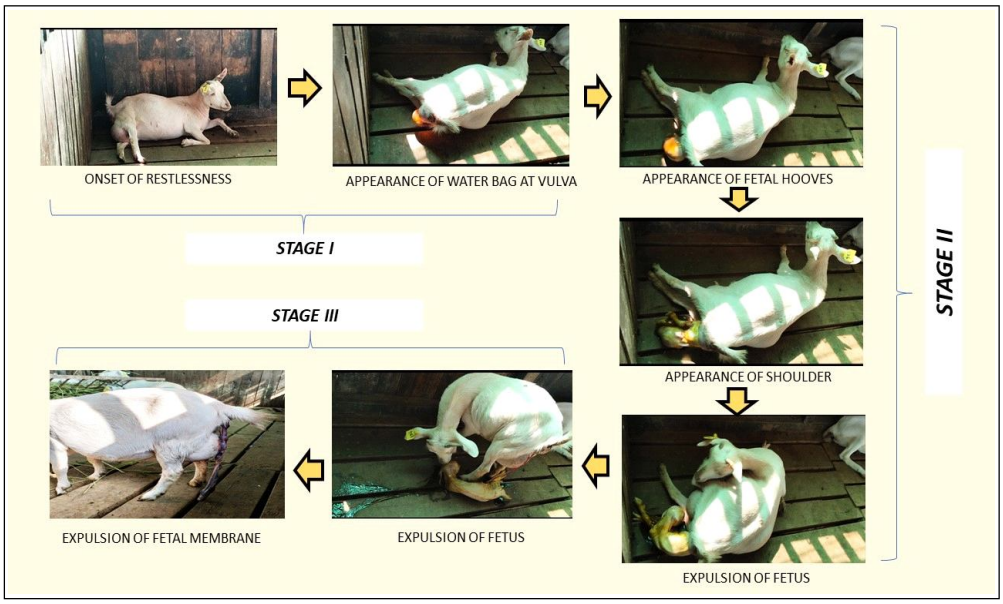


Fig 5: Stages of parturition.

between the does using the SPSS (version 20.0, SPSS, Chicago, IL, USA). Difference with values of P≤0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Interval between kidding and occurrence of first postpartum oestrus

The mean interval between kidding and occurrence of first postpartum oestrus in Assam Hill goat supplemented with different doses of nano zinc are presented in Table 1. In the present study the shortest interval from kidding to first postpartum oestrus was found to be in 50 mg nano zinc/kg concentrate mixture supplemented group and the longest in control. Sadat (2014) observed the mean interval from kidding to first oestrus as 101 days in Djallonke sheep which was similar with the control group of our present study. In contrary to the present study Kunbhar *et al.* (2016) reported slightly shorter postpartum oestrus period (68.21±0.46 days) in Kamhori goat. Zeedan *et al*. (2009) reported improvement in shortening of postpartum oestrus interval in buffaloes supplemented with Biogen-zinc and reported zinc has a critical role in the repair and maintenance of the uterine lining following parturition, speeding return to normal reproductive function and oestrus (Zeedan *et al*., 2009). The findings of our present study might be due to influence of nano zinc.

Duration of oestrus

The mean duration of oestrus in the present study are presented in Table 1. Similar findings were reported by Pietroski *et al*. (2013) in Saanen goats and Hashem and Sallam (2019) in Zaraiebi goats. However longer duration of oestrus was reported by Islam, (2003) in Beetal goats (36.21±0.91) and Assam local goats (34.18±0.76) hours. However, shorter oestrus duration was reported by Barua *et al*. (2000) in Assam local goats (20.56±0.16) hours and Fonseca *et al*. (2005) in native goats of Brazil (19.8±10.6) hours. The variation of the present finding from that of other workers might be due to the influence of various intrinsic and extrinsic factors such as methods of oestrus detection (Kanai and Shimizu, 1983), effect of breeds or strains, age, geographical location (Evans and Maxwell, 1988), climatic conditions and endocrine profile of the animal at oestrus.

Intensity of oestrus

In the present study the frequency of occurrence of intermediate oestrus was higher (66.66%) followed by intense and weak oestrus among all the treatment and control group (Table 1). The variation of the present finding might be due to nano zinc supplementation at different doses to the animals as because generally the intensity of oestrus is poor in this breed of goat.

Behavioural signs of oestrus

Bleating (100.00%) and wagging of tail (66.66 to 100.00%) were the most common behavioural signs of oestrus in all the groups of Assam Hill goats. Although the other behavioural signs observed during oestrus in all the groups of animal are presented in Table 2. Almost similar observation on the occurrence of bleating during oestrus was reported by Bhattacharyya *et al*. (2000) in Assam local goats (range 84.00 to 92.68%) and lower frequency of occurrence of bleating during oestrus was reported by Islam (2003) in Assam local goats range (28.57 to 53.58%). The highest frequency percentage of wagging of tail, restlessness and stand to be mounted were observed during oestrus in 100.00 per cent animals among the group. Similar observation on the frequency of occurrence of wagging of tail was reported by Islam (2003) in Assam local goats (94.64%). The signs of oestrus recorded in the present study

were also reported by Sankar Ganesh *et al*. (2014) in native goats and Dogra *et al*. (2017) in Beetal goats. The difference of extent in respect of occurrence of various behavioural signs of oestrus in goats could be due to variation of individual endocrine profile, breed, climate and management (Pandey and Ambatkar, 1991).

Physical signs of oestrus

The physical sign of oestrus of does are presented in Table 3. Clear and scanty vaginal mucus was found to increase in frequency percentage from 66.66 to 100.00 and 83.33 to 100.00 respectively among the group. Almost similar occurrence (80.00%) was recorded by Barua *et al*. (2000). On the contrary, Bhattacharyya *et al*. (2000) recorded lowest (39.02%) oestrual discharge in Assam local goats. Islam (2003) recorded 64.29 per cent and 54.55 per cent of scanty oestrual discharge in Assam local goats and Beetal goats respectively. Similar findings of clear vaginal mucus was observed by Dogra *et al*. (2017) in Beetal goats, while slightly lower occurrence of clear vaginal mucus (57.14%) was observed by Islam (2003) in Assam local goats. Whereas in the present study, only 16.66 to 33.33 per cent does showed opaque vaginal mucus in all the groups. Consistency of vaginal mucus was found to be thin in 16.66 to 50.00 per cent in all the group of Assam Hill goats. Thick vaginal mucus was observed in 50.00 to 83.33 per cent of Assam Hill goats

Table 1: Interval between kidding and occurrence of first postpartum (PP) oestrus (days), Average duration of oestrus (hour) and intensity of oestrus as intense, intermediate and weak (%) in different groups of nano zinc supplemented Assam hill goat.

Reproductive parameter	Groups			
	T0	T1	T2	T3
Interval between kidding and first PP Oestrus (Days)	107.17±2.42^{a}	98.83±0.70^{b}	86.50±0.62^{c}	69.00±1.57^{d}
Duration of oestrus (hour)	29.67±1.82	26.33±0.99	28.67±1.69	25.00±1.39
Intense oestrus (%)	33.33	16.66	33.33	16.66
Intermediate oestrus (%)	50.00	66.66	66.66	50.00
Weak oestrus (%)	16.66	16.66	00.00	33.33

Means bearing different superscripts differ significantly (P<0.05).

Table 2: Frequency of occurrence of different behavioural signs of oestrus in different groups of Asaam hill goat (n=6).

Behavioural signs	Frequency (%)			
	Groups			
	T0	T1	T2	T3
Bleating	100.00	100.00	100.00	100.00
Restlessness	83.33	66.66	100.00	50.00
Seeking male	66.66	50.00	83.33	33.33
Wagging of tail	100.00	83.33	100.00	66.66
Stand to be mounted	83.33	50.00	100.00	50.00

Table 3: Frequency of occurrence of different physical signs of oestrus in different groups of Assam hill goat (n=6).

Physical signs		Frequency (%)			
		Groups			
		T0	T1	T2	T3
Quantity of vaginal mucus	Scanty	100.00	83.33	100.00	83.33
Colour of vaginal mucus	Clear	83.33	66.66	100.00	66.66
	Opaque	16.66	33.33	00.00	33.33
Consistency of vaginal mucus	Thin	33.33	50.00	16.66	50.00
	Thick	66.66	50.00	83.33	50.00
Colour of vulvar mucus membrane	Pink	100.00	83.33	100.00	66.66
	Pale	00.00	16.66	00.00	33.33
Spinnbarkeit test	High	50.00	33.33	50.00	00.00
	Medium	33.33	50.00	33.33	66.66
	Low	16.66	16.66	16.66	33.33
Arborization pattern	Typical	50.00	33.33	66.66	33.33
	Atypical	50.00	50.00	33.33	33.33
	Nil	00.00	16.66	00.00	33.33

in all the groups. Similar findings of thick vaginal mucus (57.14%) was observed by Islam (2003) in Assam local goats. In the present study, the other common physical signs of oestrus were observed as pink vulvar mucosa, low to high degree of spinnbarkeit and presence of typical to atypical fern pattern arborization (Table 3). Colour of vulvar mucus membrane was observed as pink in 66.66 to 100.00 per cent does in all the groups of Assam Hill goat. Similar finding was reported by Islam (2003) who observed pink vulvar mucosa (85.71%) in Assam local goats (77.27%) and in Beetal goats. Dogra *et al*. (2017) reported clear cervical mucus with high degree of spinnbarkeit and arborization in majority of the Beetal goats.

Conception rate

Conception rates based on supplementation of nano zinc at different does are presented in Table 4. The conception rate recorded pertaining to 35 mg/kg and 50 mg/kg nano zinc supplemented does in the present study was 83.33 per cent. The present finding is in agreement with El-Nour *et al*. (2010) who reported 80.00 per cent conception rate in zinc-methionine supplemented Baladi does. Shareef, (2021) recorded 83.50 per cent conception rate in yeast with zinc supplemented local Iraqi female goats. In contrary to the present study Kundu *et al*. (2014) observed slightly higher (100.00%) conception rate in 100 ppm zinc oxide supplemented does whereas 66.66 per cent in 50 ppm zinc oxide supplemented does. Higher per cent conception rate in the treatment group compared to control might be due to the influence of nano zinc supplementation as compared to control. Zinc is a co-factor of many enzymes and these enzymes affects vitamin A synthesis along with many body metabolism which correlates directly to improve conception rates (El-Nour *et al*., 2010).

Duration of different stages (minutes) of parturition in different groups of Assam Hill goat

The duration of stage I, stage II, stage III of parturition and total time taken for parturition are presented in Table 5. The duration of different stages of parturition obtained in the present study was similar to the findings of Islam (2003) in Assam local goats. However, Ghosh and Das (2000) and Yilmaz *et al*. (2012) obtained slightly longer duration of stage III in Black Bengal and Norduz goats respectively. As reported by Ghosh and Das (2000) total time requirement for parturition in Black Bengal goat was as more as 241.7±10.41 minutes in comparison to the present study. Though the values were found within the normal range, slight differences among various data reported by other workers might be due to breed, individual variation and body condition status of the animal.

Placental weight

The mean weight of placenta are presented in Table 6. The mean weight of placenta in the present study was found to be in close accordance to that reported by Islam (2003) in Assam local goats (268.62±9.78 g). However, Ocak and Onder (2011) reported higher placental weight in Saanen, German Fawn and Damascus goats as 583.33±45.91, 750.00±66.98 and 575.00±28.35 g respectively. Higher placental weight was recorded by Ocak *et al*. (2015) (525.54±19.65 g) and Kandemir *et al*. (2021) (432.76±48.61 g) in native goats and Sannen goats respectively.

Number of cotyledons

The mean total number of cotyledons are presented in Table 6. Almost similar figure for total number of cotyledon was reported by Islam, (2003) as 108.20±3.64 and 97.00±3.19

Table 4: Conception rate in different nano zinc supplemented groups of Assam hill goat (n=6).

No. of animals conceived	Groups			
	T0	T1	T2	T3
Per cent	4	4	5	5
	66.66	66.66	83.33	83.33

Table 5: Duration of different stages of parturition (minutes) in different groups of Assam hill goat.

Treatment	Stage 1	Stage 2	Stage 3	Total
T0	84.33±8.61	14.00±1.46	100.00±10.57	198.00±12.23
T1	86.17±12.48	17.50±2.05	105.50±10.83	209.17±18.16
T2	84.17±12.54	14.33±2.26	100.83±7.90	199.33±12.49
T3	85.33±9.13	17.50±2.62	102.50±13.59	205.33±14.99

Table 6: Weight of placenta (gram) and number of cotyledons in different groups of nano zinc supplemented Assam hill goat.

No. of cotyledons	Groups			
	T0	T1	T2	T3
Weight of placenta	247.66±3.80	251.00±4.41	246.83±3.92	249.50±3.84
	98.83±3.16	96.17±2.14	99.33±3.33	98.50±3.30

in Beetal and Assam local goats respectively and Ocak and Onder (2011) as 103.75±4.03 in Damascus goats. Ocak and Onder (2011) and Kandemir *et al*. (2021) recorded lower number of cotyledons in German Fawn and Sannen goats. Higher total number of cotyledons obtained by Ocak and Onder (2011) as 112.00±6.22 and Ocak *et al*. (2015) as 137.27±10.63 in Saanen and native goats respectively. However, higher placental weight, cotyledon number, length and density are increased with higher parity and differs among various breeds (Ocak and Onder, 2011).

CONCLUSION

Supplementation of 50 mg nano zinc to the basal diet of postpartum Assam Hill Goat significantly improved the reproductive performance by decreasing postpartum oestrus interval and increasing the conception rate.

Conflict of interest

All authors declare that they have no conflict of interest.

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