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In vivo Fertility Assessment of Frozen Thawed Pantja Buck Semen

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ABSTRACT

Background: Pantja is a medium sized, dual purpose *Tarai* goat breed of Uttarakhand and adjacent area of Uttar Pradesh. Due to the lack of pure breeding buck and uncontrolled cross breeding, population of this breed is reducing continuously. Therefore, for preservation and dissemination of this pure breed, cryopreservation of semen and artificial insemination by using frozen thawed semen can play a crucial role.

Methods: Semen samples were collected from four sexually matured Pantja bucks and after preliminary evaluation extended in tris egg yolk based extender. Post thaw semen was examined for microscopical and certain biochemical evaluation. A total 12 does were inseminated and pregnancy diagnosis was conducted at 45 days onwards by ultrasonography.

Result: Post thaw seminal attributes were significantly ($P \leq 0.01$) reduced in comparision to freshly diluted semen. Total six does (50.00%) were pregnant and five (83.33%) of them gave birth to healthy kids. Among these five, three does (60.00%) gave birth to single and two does (40.00%) gave birth to twins.

Key words: Kidding, Pantja, Post thaw, Tris egg yolk.

INTRODUCTION

Goat farming acts as an important tool in socio-economic development of rural small farmers because of easy adaptability of this animal species in adverse climatic and nutritional conditions. Nowadays, the status of goat farming has improved due to high demand of goat milk for its medicinal and nutritional values (Liang and Paengkoum, 2019). As goat meat (chevon) is free from religious taboos, it is preferred over other meats in several countries including India. Total annual meat production of the country is 8.11 million tonnes and goat meat contributes 13.53% of national meat production (Basic Animal Husbandry Statistic, 2019). According to 20th livestock census of India, there are 32.10 million of male goat in our country which is 14.65% lesser than their population in 2012 (37.62 million), whereas, there is 19.71% increase in female population (116.78 million) than 19th census (97.56 million) (Livestock Census-2019, Govt. of India). Therefore, cryopreservation of high quality goat semen and artificial insemination can help reverse the trend of negative selection by preserving pure and superior germplasm. This will ensure that only the best quality genes are passed on to the next generation, thus, improving the overall quality of the goat population. In India, the major portion of goat farming is extensive and breeding system follows uncontrolled natural mating. Detection of superior breeding buck is challenging for the goat farmers (Alkass *et al*., 1982) and small flock holders are not maintaining the breeding males as they are completely dependent upon the large flock holders who generally maintain breeding males (Goel *et al*., 2016). For better breeding management, there should be sufficient good quality stud bucks, however, it is unfortunate that due to lack of breeding bucks more than 30% estrus does remain without service (Karim *et al*., 2019).

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Pantja is a medium sized, dual purpose *Tarai* goat breed of Uttarakhand having morphological similarities with deer and commonly found in *Tarai* region of Uttarakhand and adjoining areas of Uttar Pradesh (Dhara *et al*., 2022a; Dhara *et al*., 2022b). This breed contributes about 21% of goat population in Uttarakhand and are resistant to many of the diseases as compared to other breeds (Nidhi, 2014). For augmentation and preservation of pure germplasm of this breed, artificial insemination with cryopreserved semen of same breed is need of the hour. Therefore, the present study was undertaken to cryopreserve the Pantja goat semen and further utilization of it for artificial insemination.

MATERIALS AND METHODS

The present study was conducted in the Department of Veterinary Gynaecology and Obstetrics, Govind Ballabh

Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India. Semen samples were collected twice a week in early morning by using artificial vagina (AV) method from four sexually matured Pantja bucks (weighing 30-40 kg) of about 2 to 3 years of age maintained at Goat Unit, Department of Livestock Production Management, (GBPUAT, Pantnagar). The bucks were trained for semen collection a month before starting of the experiment by AV method. The AV used was 20 cm in length and 4.5 cm in diameter and was imported from IMV, France. The AV was filled with warm water (50°C) to achieve a temperature of 43°- 45°C at the time of collection. Air was filled between hard cylinder and inner liner by manual air blower to maintain the pressure same as female genitalia. Sterilized liquid paraffin was applied to the mouth of the prepared AV for lubrication. Before collection of the semen, prepuce of the buck was washed with normal gentamicin solution (50 mg in 100 ml of water) to prevent the contamination with dirt, dust and microorganisms. Two to three false mounts were given for clearance of the urethral passage through the secretion of the Bulbourethral gland. Immediately after collection, the semen samples were taken to the laboratory and kept within a water bath at 37°C during the time of evaluation. Semen samples with mass motility (≥3+), individual motility (≥80%) and concentration (≥2×10^9/ml) and total abnormalities (<10%) were selected for further processing.

Fig 1: Artificial insemination in Pantja goat.

After initial evaluation, the suitable semen samples were pooled and diluted in egg yolk tris (EYT) extender (Tris 3.634 g, Fructose 1.259 g, Citric acid 1.990 g, egg yolk 7.5 ml, glycerol 5 ml, penicillin G 100000 IU, streptomycin 100000 µg and distilled water up to 100 ml) to reach the final concentration of 100 × 10^6/ml. The diluted semen was filled and sealed in 0.25 ml French mini straws (IMV). The straws were equilibrated at 5°C for 4 h followed by vapor freezing above 4 cm of liquid nitrogen (LN_2) level for 10 minutes. Finally, the straws were plunged at -196°C in LN_2 tank. The straws were thawed at 37°C in water bath for 30 sec and used for post-thaw evaluation and *in vivo* fertility test.

The post thaw semen samples were evaluated microscopically for progressive motility (Singh *et al*., 2013), viability (Memon *et al*., 2012), hypo osmotic swelling test (HOST) (Zubair *et al*., 2013), acrosome integrity (Watson, 1975) and total sperm abnormalities (Memon *et al*., 2013) as per standard protocol. Seminal plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated spectrophotometrically using commercial kits. Malondialdehyde (MDA) concentration was evaluated by thiobarbituric acid (TBA) test (Ducha *et al*., 2020) and glutathione peroxidase was estimated as per Wheeler *et al*. (1990).

A total of 12 does were inseminated from a period December to March to perform the *in-vivo* fertility test. The does were observed twice a day (early morning and evening) by using teasing buck to detect the estrus. The does showing heat symptoms were separated from rest of the flock and inseminated 12 hours after the detection of heat by vaginal speculum method (Fig 1). Pregnancy diagnoses of the inseminated does were performed by ultrasonography after 40 to 45 days of insemination.

The statistical analysis was done by using SPSS 16.0 software for two way analysis of variance (ANOVA). The fertility parameters were presented as percentage. All the data is presented as Mean±SE.

RESULTS AND DISCUSSION

Microscopic seminal attributes of Pantja buck semen before freezing and after thawing are depicted in Table 1. Each of

Table 1: Physical characteristics of post-thaw Pantja buck semen.

Parameters	Pre-freeze	Post-thaw	P-value	Percent changes
Progressive motility (%)	85.38±2.70^b	29.75±1.87^a	0.00	65.16
Viability (%)	84.75±3.43^b	38.64±1.93^a	0.00	54.41
HOST (%)	87.74±1.58^b	39.21±2.00^a	0.00	55.31
Acrosome integrity (%)	94.74±1.89^b	61.55±1.39^a	0.00	35.03
Total sperm abnormality (%)	7.95±1.13^a	10.71±1.34^b	0.00	34.72

Mean values with different superscript in a row differ significantly (P≤0.01).

HOST- Hypo osmotic swelling test, ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, MDA- Malondialdehyde, GSH-Px- Glutathione peroxidase.

Table 2: Biochemical attributes of post-thaw Pantja buck semen.

Parameters	Pre-freeze	Post-thaw	P-value	Per cent changes
ALT (U/L)	18.10±1.20[a]	232.88±4.70[b]	0.00	92.23
AST (U/L)	124.04±8.48[a]	295.44±14.33[b]	0.00	51.02
MDA (nmol/ml)	2.14±0.09[a]	8.04±0.39[b]	0.00	73.38
GSH-Px (U/ml)	10.90±0.53[b]	2.75±0.33[a]	0.00	74.77

Mean values with different superscript in a row differ significantly (P≤0.01).

ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, MDA- Malondialdehyde, GSH-Px- Glutathione peroxidase.

Table 3: *In vivo* fertility parameters of cryo-preserved Pantja buck semen following artificial insemination.

Parameters	Values
Number of does inseminated	12
Number of does conceived [n (%)]	6 (50.00)
Number of does kidding [n (%)]	5 (83.33)
Number of does kidding single [n (%)]	3 (60.00)
Number of does kidding twins [n (%)]	2 (40.00)

the microscopic seminal attributes showed significant (P≤0.01) reduction in their values at post thaw as compared to pre-freeze stage. Our findings are in agreement with the studies of previous workers in different goat breeds. These significant changes in seminal attributes may be due to cryo-injuries developed at the time of freezing-thawing. The freezing-thawing process leads to marked cellular and functional disruption of sperm plasma and acrosomal membrane due to the formation of intracellular ice crystals and an increased concentration of solutes (Susilowati *et al*., 2019). In addition, the cryopreservation process causes nuclear damage and DNA disintegration due to the production of reactive oxygen species (ROS), which ultimately leads to lipid peroxidation, membrane damage and death of the spermatozoa (Zamiri, 2020).

Mean value of post thaw progressive sperm motility (29.75±1.87) in the present study was higher than the findings of Ramachandran *et al*. (2015) in Jamunapari bucks (28.82±1.99) and Tabarez *et al*. (2017) in Blanca de Rasquera bucks (18.2-24.5) and lesser than Black Bengal bucks (40.89±0.65) (Singh *et al*., 2016), Alpine Sannen and Beetal crosses (45-56) (Narwade *et al*., 2017).

Mean percentage of sperm viability (38.64±1.93) in the present study was higher than as observed in Boer bucks (27.33±0.85) (Yodmingkwan *et al*., 2016) and lower than Jamunapari bucks (41.01±3.02) (Ramachandran *et al*., 2015), Gaddi bucks (45.26±1.32) (Sharma, 2018) and Black Bengal bucks (50.48±0.65) (Singh *et al*., 2016).

The mean values of per cent HOST-reactive spermatozoa (39.21±2.00) in present study were higher than Boer bucks (8.04±1.02) (Yodmingkwan *et al*., 2016), however, lower than Jamunapari bucks (41.01±3.02) (Ramachandran *et al*., 2015); Black Bengal bucks (47.02±0.58) (Singh *et al*., 2016); Gaddi bucks (52.48±1.43) (Sharma, 2018) and Alpine Sannen and Beetal crosses (61.0-64.63) (Narwade *et al*., 2017).

The mean percentage of sperm total morphological abnormalities (10.71±1.34) in the present study were found lower than as reported by Singh *et al*. (2016) in Black Bengal bucks (13.37-16.81); Thakur *et al*. (2005) in Chegu bucks (23.3) and higher than as reported by Yodmingkwan *et al*. (2016) in Boer bucks (1.54-1.71); Ramachandran *et al*. (2015) in Jamunapari buck (2.84±0.49) and Sharma (2018) in Gaddi bucks (2.84±0.49).

Mean values of seminal plasma transaminase enzymes (AST, ALT), glutathione peroxidase and lipid peroxidation (MDA) in fresh and frozen thawed semen are presented in Table 2. Sharma *et al*. (2013) recorded 115.79; 71.93; 52.63 and 107.02 per cent increased level of GPT activity and 111.96; 84.54; 72.69 and 98.08 per cent elevation of GOT activity in post-thawed semen compared to fresh semen at various cooling rate (*i.e.* 15, 20, 25 and 30°/min.). Present findings are in accordance with the finding of Hussain *et al*. (2016) who found eight fold increase in level of GPT and GOT activity in Holstein bull semen at post-thawed stage compared to fresh semen. This variation in post thaw transaminase activity observed by different researchers may be due to breed difference and season variation (Chaudhary and Sadhu, 1976).

Higher value of post thaw glutathione peroxidase activity in ram semen has been observed by Mehdipour *et al*. (2017) who reported 11.6 U/ml of GSH-Px activity in soybean lecithin based dilutor. Hu *et al*. (2010) reported 166.18±18.67 U/ml of GSH-Px activity in bovine frozen thawed semen. Kumar (2010) found glutathione peroxidase activity in Barbari buck semen supplemented with Zn and Se after cryopreservation at days 0, 60, 75, 90 and 105 were 4.42±0.23 U/ml, 4.3±0.51 U/ml, 4.83±0.17 U/ml, 4.86±0.11 U/ml, 4.92±0.18 U/ml (control) and 5.7±0.52 U/ml, 7.9±0.13 U/ml, 8.34±0.19 U/ml, 9.46±0.25 U/ml, 10.01±0.12 U/ml (supplemented groups), respectively. In case of Dog, the value of GSH-Px was documented as 237.6±18.4 after 3 hours of freezing, 216.8±4.3 after 24 h of freezing, 219.3±4.5 Units/50 × 10^6 spermatozoa (Chatdarong *et al*., 2012). This difference in the findings by other workers may be due to difference of species, breed, freezing technique and types of extender.

MDA represents the level of lipid peroxidation (Hsieh *et al*., 2006). Prolonged lipid peroxidation is also an indication of plasma membrane damage due to changes in the lipid matrix structure (Ducha, 2018). MDA is also negatively correlated with the spermatozoa motility (Hsieh *et al*., 2006).

Conception rate and other fertility parameters of frozen thawed Pantja buck semen post insemination are presented

in Table 3. Kharche *et al*. (2013) reported 53.12 per cent pregnancy rate and 44.44 per cent kidding rate in case of Jamunapari goats, inseminated with frozen semen diluted by egg yolk based extender. Present findings are in agreement with Yotov *et al*. (2016), who reported 33.3 per cent conception rate in Bulgarian white milk goat, in case of natural estrus with single dose inseminated. According to Central Institute for Research on Goats (CIRG) Annual Report (2017), 37.57 per cent kidding rate was found in different goat breeds, inseminated with egg yolk based extender. Karunakaran *et al*. (2018) reported 47.26 per cent kidding rate in case of Black Bengal goat inseminated after 12 hours of estrus detection with egg yolk based extender. Sharma and Sood (2019) reported 42.5 per cent conception rate with a twinning rate 11.8 per cent in Chegu goats by using frozen thawed semen diluted with 10 percent egg yolk based extender. Andrabi *et al*. (2017) artificially inseminated Beetal (n=23) and Jattal (n=54) goats using frozen-thawed semen after 12 hrs of heat detection and a conception rate of 50 per cent and 34 per cent was recorded in both breeds, respectively with an overall pregnancy rate of 42 per cent.

CONCLUSION

Semen cryopreservation and artificial insemination by using frozen thawed semen can successfully become a tool for preservation and dissemination of pure germ plasm of indigenous Pantja goat breed. However, more studies are required about large numbers of field fertility evaluation.

Conflict of interest: None.

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