

Studies on fresh and cryopreserved seminal attributes in Beetal bucks outside their breeding tract

Abstract

The objectives of the present research were to assess fresh and cryopreserved seminal characteristics of breeding Beetal buck semen outside of its breeding tract. Semen was collected twice in a week from twelve Beetal bucks for 6 weeks. The mean value of ejaculate volume(ml), mass motility(o-5 scale), progressive motility(%), sperm viability (%), intact acrosome(%), sperm abnormality(%) and plasma membrane integrity(%) were ranged from 0.73 ± 0.08 ml to 1.17 ± 0.27 ml; 4.17 to 4.67 , 80.83 ± 1.54 to 87.50 ± 2.14 ; 83.83 ± 0.95 to 90.33 ± 0.80 ; 87.00 ± 0.86 to 94.67 ± 0.33 ; 6.83 ± 0.31 to 10.33 ± 0.67 and 59.33 ± 1.52 to 66.67 ± 1.17 , respectively. The mean value of post thaw sperm motility, post thaw sperm viability, post thaw acrosome integrity, post thaw sperm abnormalities and post thaw plasma membrane integrity were ranged from 52.50 ± 1.12 to 66.67 ± 1.67 ; 60.00 ± 1.24 to 69.67 ± 1.05 ; 66.00 ± 0.77 to 78.83 ± 2.15 ; 9.00 ± 0.45 to 12.83 ± 0.60 and 41.50 ± 1.18 to 53.50 ± 1.23 , respectively. It was determined that, even outside of the breeding tract, the foundational characteristics of Beetal bucks are within the range defined for other Indian breeds of goat.

Keywords: Beetal buck, cryopreservation, semen parameter, sperm viability

1. Introduction

According to Monteiro et al. (2018), goats (*Capra hircus*) are among the first animals to be domesticated and have been around for at least 10,000 years. They are distributed around the world as a result of their ability to adapt to various climatic and environmental circumstances (Mahmoud, 2010). According to Sinn and Rudenberg (2008), goats are the most useful animals on the planet since they can produce meat, milk, fiber, fertilizer, and draft power. According to data from the 20th Livestock Census of 2019, there are 148.88 million goats in the nation, or 27.8% of all livestock. 6.16 million goats were counted in Assam as of the 20th livestock census in 2019. Compared to other livestock species, goats offer unique social, economic, managerial, and biological benefits. They also make a substantial economic contribution to the agrarian economy.

In commercial goat farms, the Beetal breed of goats is thought to be a very profitable breed to raise. This breed's milk production ranks second, after Jamunapuri. The Beetal goat breed serves both dairy and meat purposes. Their skin is excellent enough to be used in the production of leather goods that are in high demand.

Research on the quality and freezability of semen is crucial for the preservation of Beetal buck germplasm, but studies on seminal characteristics and freezability in this species are insufficient and preliminary. Hence, the present investigation was carried out to evaluate fresh and cryopreserved seminal attributes of the Beetal buck outside its breeding tract.

2. Materials and Methods

2.1 Experimental animals

Twelve adult Beetal bucks maintained under an intensive system of management at Goat Research Station, Assam Agricultural University, Burnihat, Kamrup Metro, Assam, India were used in the present study. After the bucks' general and sexual health was assessed, they were chosen for the current investigation. The chosen bucks were immunized, dewormed, and in good health. During the study period, the bucks were kept under consistent feeding and management practices, with minimal grazing during stall feeding.

2.2 Semen collection

Using a standard artificial vagina (at 42°C) and a restrained doe as a mount, semen was collected aseptically and hygienically from each buck once or twice a week in the morning. The study used a total of 72 ejaculates, six of which came from each buck.

2.3 Evaluation of Semen

Semen Evaluations were carried out for different seminal characteristics, such as volume, mass activity (Zemjanis, 1970), individual motility, percentage of live sperms (Blom, 1977), percentage of total abnormal sperms (William, 1920), percentage of intact acrosome (Watson and Martin, 1975) and Hypo-osmotic swelling test (Revell and Mrode, 1994).

The semen samples were cryopreserved, and after 24 hrs, post-thaw semen characteristics were evaluated. Following the tabulation of the experimentation data, statistical analysis was conducted.

2.4 Statistical analysis

With the use of SPSS software (ver. 20), data on seminal profiles of Beetal buck and semen characteristics were evaluated by one way analysis of variance using a general linear model. The post-hoc Tukey test was used to compare mean values that showed significant differences.

3. Results and Discussion

3.1 Fresh Semen characteristics

3.1.1 Ejaculate volume

The mean ejaculate volume ranged from 0.73 ± 0.08 ml to 1.17 ± 0.27 ml. Analysis of variance revealed that the mean ejaculate volume did not differ significantly ($p > 0.05$) between the animals. Similar results were also reported by Goswami *et al.* (2020) in the Beetal buck (1.08 ± 0.05 ml). Ejaculate volume reported by Dhillon *et al.* (2020) was slightly higher (1.2 ± 0.1 ml) than that reported in the present study. Ejaculate volume reported by Narwade *et al.* (2018) in crossbreed buck (Saanen \times Beetal) and Atara *et al.* (2018) in Surti buck were similar to the present study.

3.1.2 Mass motility

Mean mass activity was ranging from 4.17+ to 4.67+ in the Beetal buck semen. Analysis of variance revealed that there was no significant difference ($p > 0.05$) in the mean mass activity between animals. The mass motility of Beetal buck semen recorded in the present study was

higher than the values recorded by earlier workers in Beetal buck (Dutta Borah, 2005; Akela, 2006; Das, 2007; Sarma et al., 2011; Goswami, 2014). The variation in the mass motility may be due to variation in the quality of the semen, season and microscopic examination technique of the individual observer (Bhakat et al., 2015).

3.1.3 Progressive motility

The mean per cent individual progressive motility ranged from 80.83 ± 1.54 to 87.50 ± 2.14 in Beetal bucks semen. Analysis of variance revealed that there was no significant difference ($p > 0.05$) in the mean individual progressive motility between animals. Similar results were also reported by Sarma et al., (2011) in Beetal buck ($86.03 \pm 0.87\%$). Almost similar values ($82.94 \pm 0.73\%$), ($85.73 \pm 0.43\%$) and ($87.20 \pm 0.41\%$) to that in the present study were also reported by Goswami et al. (2020), Parmar et al., (2011) and Borgohain (1981), respectively. Dhillon et al. (2020), Deori et al. (2018) and Sultana et al. (2013) reported a lower sperm motility per cent of 71.1 ± 6.9 in Beetal buck, 77.97 ± 0.73 in Assam Hill Goat buck and 77.07 ± 1.06 in Black Bengal buck, respectively.

3.1.4 Sperm Concentration

The mean sperm concentration ranged from 2429.00 ± 40.38 to 2871.00 ± 51.00 million/ml, respectively. Analysis of variance revealed that there was a highly significant ($p < 0.01$) difference in the mean sperm concentration in semen between the animals. The mean sperm concentration (million/ml) recorded in the present study was similar to that reported by Moce et al. (2022), Sultana et al. (2013) and Aguiar et al. (2013) which was 2609 ± 193 , 2827 ± 0.76 and 2500 ± 0.1 , respectively.

3.1.5 Sperm viability

The mean per cent sperm viability of the Beetal buck semen were ranged from 83.83 ± 0.95 to 90.33 ± 0.80 . Analysis of variance revealed that there was a highly significant ($p < 0.01$) difference in the mean sperm viability between the animals. The mean per cent sperm viability observed in the current study was similar to that reported by Goswami et al. (2020) in Beetal buck, Siddiqua et al. (2016) in Black Bengal goat, Deori et al. (2018) in Assam Hill goat, Sultana et al. (2013) in Black Bengal buck and Duchá et al. (2021) in Senduro buck

3.1.6 Sperm abnormalities

The mean per cent sperm abnormalities ranged from 6.83 ± 0.31 to 10.33 ± 0.67 in the present study, which is similar to that recorded by Deori et al. (2018) in Assam Hill Goat buck (7.66 ± 0.73). The mean per cent sperm abnormalities recorded in the present study was higher than that reported by Goswami et al. (2020) in Beetal buck (5.92 ± 0.48), Atara et al. (2018) in Surti buck (2.57 ± 0.12), Sultana et al. (2013) in Black Bengal buck (8.05 ± 0.26) and Ahmad et al. (2014) in Beetal buck (3.2 ± 0.64).

3.1.7 Plasma membrane integrity (HOST)

The range of mean per cent HOST reacted sperm observed in the current study was 59.33 ± 1.52 to 66.67 ± 1.17 , which was similar to that recorded by Deori et al. (2018) in Assam Hill Goat buck (66.95 ± 0.74) but higher than that reported by Moce et al. (2022) in buck (54 ± 0.2). However, Kumar et al. (2014) reported a comparatively higher value in Black Bengal buck during spring (82.77 ± 0.27) and summer (76.66 ± 2.35) and Sarma et al. (2011) in Beetal buck (83.58 ± 1.01).

3.1.8 Acrosome integrity

The mean per cent acrosome integrity ranged from 87.00 ± 0.86 to 94.67 ± 0.33 in the present study, which is similar to that reported by Ahmad et al. (2014) in Beetal buck (87.7 ± 1.3) and Deori et al. (2018) in Assam Hill Goat buck (93.34 ± 0.51). The mean per cent acrosome integrity recorded in the present study was higher than that observed by Goswami et al. (2020) in Beetal buck, Dhillon et al. (2020) in Beetal buck and Moce et al. (2022) in buck, which was 85.18 ± 0.39 , 80.4 ± 1.2 and 56 ± 3.7 , respectively.

The genetic composition of each individual buck, age, body weight, scrotal circumference, semen collection techniques used at the time of measurement, and other factors could all contribute to variations in semen characteristics (Bhakat et al., 2015).

3.2 Cryopreserved semen characteristics

Cryopreservation of caprine semen includes dilution, cooling, freezing, storing, and thawing of straws.

3.2.1 Post thaw sperm motility

The range of mean per cent post thaw sperm motility was 52.50 ± 1.12 to 66.67 ± 1.67 in the present study. Similar results were also recorded by Morrell et al. (2022), Karim et al. (2018) in Black Bengal buck, Chakravarty (2019) in Beetal buck, Baruah (2018) in Beetal buck, Deori et al. (2018) in Assam Hill buck and Dutta (2021) in Assam Hill Goat which was 55 ± 21 , 55.63 ± 3.42 , 63.69 ± 0.44 , 62.05 ± 0.76 , 55.39 ± 0.97 and 58.97 ± 0.32 per cent post thaw sperm motility, respectively.

3.2.1 Post thaw sperm viability

The range of mean per cent post thaw sperm viability was 60.00 ± 1.24 to 69.67 ± 1.05 in the current study. Similar results were recorded by Chakravarty (2019) in Beetal buck semen in Tris extender ($67.62 \pm 0.35\%$), Baruah (2018) in Beetal buck semen in Tris extender ($66.40 \pm 0.94\%$) and Deori et al. (2018) in Assam Hill goat semen (71.01 ± 0.78). The mean per cent post thaw sperm viability recorded in the present study was higher than that recorded by Naing et al. (2011) in Boer goat semen (58.32 ± 2.19), Baruah (2018) in Beetal buck semen in Tris extender containing 1.5% soya lecithin (55.67 ± 1.34), but lower than that observed by Kalita (2016) in Beetal goat semen in Tris extender (72.65 ± 0.73) and Chakravarty (2019) in Beetal buck semen in Optixcell extender (70.69 ± 0.43).

3.2.3 Post thaw acrosome integrity

The range of mean percent post thaw acrosome integrity was 66.00 ± 0.77 to 78.83 ± 2.15 in the present study. Similar results were recorded by Sahoo (2020) in Beetal buck semen extended using Optixcell (68.99 ± 1.30), Naing et al. (2011) in Boer goat semen (66.67) and Kalita (2016) in Beetal buck semen (67.50). The mean per cent post thaw acrosome integrity recorded in the present study was higher than that recorded by Sahoo (2020) in Beetal buck semen in Bioxcell (54.63 ± 1.45), Baruah (2018) in Beetal buck semen in Optixcell extender (59.98 ± 0.78) and Kakati (2016) in Beetal buck semen in Tris extender (45.07 ± 0.74).

3.2.4 Post thaw sperm abnormalities

The mean per cent post thaw sperm abnormalities ranged from 9.00 ± 0.45 to 12.83 ± 0.60 in the present study. Similar results were reported by Sharma (2018) in washed ejaculate of Gaddi buck (9.7 ± 0.9). The mean per cent post thaw sperm abnormalities recorded in the present study was lower than that recorded by Sharma (2018) in non washed ejaculate of Gaddi buck (14.3 ± 0.8).

3.2.5 Post thaw plasma membrane integrity (HOST)

The range of mean percent post thaw HOST reacted sperm was 41.50 ± 1.18 to 53.50 ± 1.23 in the present study. Similar results of mean per cent post thaw HOST reacted sperm was recorded by Chakravarty (2019) in Beetal buck semen in Tris extender (47.48 ± 0.39), Baruah (2018) in Beetal buck semen in Tris extender (51.53 ± 0.90) and Optixcell extender (50.06 ± 0.69) and Sharma (2018) in non washed ejaculate of Gaddi buck (45.6 ± 3.8). The mean per cent post thaw HOST reacted sperm in the present study was lower than that recorded by Sahoo (2020) in Beetal buck semen in Optixcell (70.43 ± 1.40) and Bioxcell (57.92 ± 1.63) and Tris extender (64.73 ± 1.78).

4. Conclusion: It can be concluded that, even outside of the breeding tract, the foundational characteristics of Beetal bucks are within the range defined for other Indian breeds of goat. This information highlights the need for more research with a bigger sample size as well as field investigations using semen to measure the conception rate and evaluate the fertility of Beetal bucks

5. References

- Aguiar, G.V.; Tilburg, M.F.V.; Catunda, A.G.V.; Celes, C.K.S.; Lima, I.C.S.; Campos, A.C.N., Moura, A.A.A. and Araújo, A.A. (2013). Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. *Brazilian Journal of Veterinary and Animal Sciences*, 65(1): 6–12.
- Ahmad, M.; Nasrullah, R.; Riaz, H.; Sattar, A. and Ahma, N. (2014). Changes in motility, morphology, plasma membrane and acrosome integrity during stages of cryopreservation of buck sperm. *Journal of the South African Veterinary Association*, 85(1): 01-04.

Akela A. (2006). Seasonal variation in semen characteristics of Assam local and Beetal bucks. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Atara, V.B.; Chaudhari, C.F.; Ramani, U.V.; Chaudhary, M.M.; Patel, D.K.; Patel, Y.R. and Patel, N.G. (2018). Semen characteristics in young and adult Surti buck. *Indian Journal of Animal Health*, 57(2): 219-224.

Baruah, D. (2018). Effect of extender and removal of seminal plasma on quality of frozen Beetal buck semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Bhakat, M.; Mohanty, T.K.; Gupta, A.K.; Prasad, S.; Chakravarty, A.K. and Khan, H.M. (2015). Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin*, 34(1): 100-112.

Bhakat, M.; Mohanty, T.K.; Gupta, A.K.; Prasad, S.; Chakravarty, A.K. and Khan, H.M. (2015). Effect of season on semen quality parameters in Murrah buffalo bulls. *Buffalo Bulletin*, 34(1): 100-112.

Blom, E. (1977). Sperm morphology with reference to bull infertility. In: *Some papers contributed to the First All India Symposium on Animal Reproduction*, Punjab Agricultural University, Ludhiana. pp. 61-81.

Borgohain, A.C. (1981). Studies on semen characteristics and fertility of Assam local and Beetal bucks. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Chakravarty, H. (2019). A study on centrifugation regime and commercial extender on quality of frozen Beetal buck semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Das, D. (2007). Physico-biochemical studies on buck semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Deori, S.; Deka, B.C.; Biswas, R.K.; Nahardeka, N.; Arangasamy, A.; Bhuyan, D.; Kalita, D.J.; Borah, R.S. and Arundhati, P. (2018). Characteristics and freezability of Assam Hill goat semen. *Indian Journal of Animal Research*, 52(1): 25-28.

Dhillon, N.; Cheema, R.; Kaswan, S. and Singal, S. (2020). Seminal attributes, antioxidant defense system and fertility rate reveal an association in Beetal goat bucks. *International Journal of Livestock Research*, 10(6): 85-96.

Ducha, N.; Budijastuti, W. and Rahayu, D.A. (2021). Senduro goat semen characteristics as a candidate for low temperature storage. *E3S Web Conf.*, 328(08010): 1627-1640.

Dutta Borah, B.K. (2005). Characteristics and preservation of Beetal buck semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Dutta, M. (2021). Characterization of membrane proteins in fresh and frozen spermatozoa in Assam Hill Goat. Ph.D. Thesis, Assam agricultural University, Khanapara, Guwahati-22.

Goswami, M. K. (2014). Studies on seminal attributes of Sirohi and Beetal bucks and effect of certain additives on the quality of frozen semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Goswami, M.K.; Sinha, S.; Deka, B.C.; Biswas, R.K. and Dutta, A. (2020). A comparative study on seminal attributes of Sirohi and Beetal bucks. *Pharma Innovation*, 9(6): 262-266.

Kakati, U. (2016). Effect of soybean lecithin based extender on quality of frozen semen in Beetal, Sirohi and Assam Hill Goat. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Kalita, M.K. (2016). Studies on effect of extenders and different sperm number per straw on quality of frozen semen in Beetal and Sirohi bucks. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.

Karim, M.F.; Khandoker, M.A.M.Y. and Husain, S.S. (2018). Fact findings about the frozen buck semen characters freezing with eyc and tris extender and productivity of Black Bengal does as the potential genetic resource in Bangladesh. *Res. Agric. Livest. Fish.* 5 (3): 341-350.

Kumar, R.; Pramod, R.K.; Kumar, R.; Negi, M.; Singh, S.P.; Singh, R. and Mitra, A. (2014). Testicular biometry and seasonal variations in semen parameters of Black Bengal goats. *Indian Journal of Animal Sciences*, 84(6): 635-639

Mahmoud, A.A. (2010). Present Status of the World Goat Populations and their Productivity. *Lohman Information*, 45(2): 42.

Mocé, M.L.; Esteve, I.C.; Pérez-Fuentes, S.; Gómez, E.A. and Mocé, E. (2022). Microbiota in Goat Buck Ejaculates Differs Between Breeding and Non-breeding Seasons. *Front. Vet. Sci.*, 9:867671

Monteiro, A.; Costa, J. M. and Lima, M. J. (2018). Goat System Productions: Advantages and Disadvantages to the Animal, Environment and Farmer. *Goat Science*

Morrell, J.M.; Malaluang, P.; Ntallaris, T. and Johannission, A. (2022). Practical method for freezing buck semen. *Animals*, 12(3): 352.

- Naing, S.W.; Haron, A.W.; Khan, M.A.; Yusodd, R.; Bakar, M.Z.A.; Sarsaifi, K. (2011). Effect of seminal plasma removal, washing solutions, and centrifugation regimes on Boer goat semen cryopreservation. *Pertanika J. Trop. Agri. Sci.*, 34: 271-279.
- Narwade, B.M.; Mohanty, T.K.; Bhakat, M.; Rahim, A.; Sinha, R, and Singh, A.K. (2018). Seasonal influence on semen production performance of crossbred buck (Saanen x Beetal) in an organized farm. *International Journal of Livestock Research*, 8(8): 196-203
- Parmar, V.R.; Suthar, B.N.; Nakhshi, H.C.; Parikh, S.S. and Chauhan, P.M. (2011). Study on physical characteristics of Mehsana buck semen. *Indian Journal of Animal Research*, 45(3): 207-210
- Revell, S.G. and Mrode, R.A. (1994). An osmotic resistance test for bovine semen. *Anim. Reprod. Sci.*, 36: 77-86
- Sahoo, B. (2020). Effect of commercial extender and curcumin as additive on quality of frozen Beetal buck semen. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.
- Sharma, A.K.(2018). Investigation on follicular dynamics and semen cryopreservation in goats. Ph.D. Thesis, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062.
- Siddiqua, A.; Islam, N.; Rahman, M.; Khandoker, M.A.M.Y.; Saleh, A. and Bari, M. (2016). Evaluation of semen quality of Black Bengal Goat in Bangladesh. *International Journal of National Society of Sciences*, 3(1): 05-09.
- Sinn, R. and P. Rudenberg (2008). Raising Goats for Milk and Meat. Little Rock, Arkansas: *Heifer International*
- Sultana, F.; Husain, S.S.; Khatun, A.; Apu, A.S. and Khandoker, M.A.M.Y. (2013). Study on buck evaluation based on semen quality and fertility. *Bangladesh Journal of Animal Science*, 42(2): 101-108.
- Sultana, F.; Husain, S.S.; Khatun, A.; Apu, A.S. and Khandoker, M.A.M.Y. (2013). Study on buck evaluation based on semen quality and fertility. *Bangladesh Journal of Animal Science*, 42(2): 101-108.
- Sultana, F.; Husain, S.S.; Khatun, A.; Apu, A.S. and Khandoker, M.A.M.Y. (2013). Study on buck evaluation based on semen quality and fertility. *Bangladesh Journal of Animal Science*, 42(2): 101-108.
- Watson, P.F. and Martin, I.C.A. (1975). Effects of egg yolk glycerol and the freezing rate on the viability and acrosomal structures of frozen spermatozoa. *Aust. J. Bio. Sci.*, 28: 153-159.

Williams, W.W. (1920). Technique of collecting semen for laboratory examination with a view of several diseased bulls. *Cornell Vet.*, 10: 87-94.

Zemjanis, R. (1970). Collection and evaluation of semen In: *Diagnostic and Therapeutic Techniques in Animal Reproduction*, 2nd Edn., Williams and Wilkins Co., Baltimore. pp. 139-155.

UNDER PEER REVIEW