Bhutan Journal of Animal Science (BJAS)
Volume 2, Issue 1, Page 14-19, 2018

Full length paper

SEMINAL ATTRIBUTES, COOLING PROCEDURE AND POST THAW QUALITY OF SEMEN OF INDIGENOUS KHARI BUCK OF NEPAL

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Abstract: The objective of the study was to understand the seminal attributes, effectiveness of cooling process and post thawed semen quality of a Nepalese indigenous Khari buck. Two Khari bucks were used for the study. Thirty-two ejaculates (16 ejaculates from each buck) were evaluated for seminal attributes of fresh semen. The mean semen volume, semen color, sperm mass activity, sperm motility, sperm viability, sperm concentration, abnormal acrosome, mid piece and tail, and abnormal head were 0.73 ml, 3.10, 3.80, 80.9%, 94.6%, 2597.0×10^6 ml^{-1}, 10.7% and 5.00%, respectively. Further, 12 ejaculates (6 ejaculates) from each buck (only having ≥0.5ml semen volume, ≥80% sperm motility, ≥90% sperm viability and $\geq2500\times10^6$ ml^{-1} sperm concentration) were considered for cooling, freezing and post thawed semen quality. Tris-egg-yolk-citrate extender was used for semen dilution. The mean sperm motility and viability of post diluted semen after 90 minutes of cooling were 73.8% and 88.1%, respectively. Similarly, the mean sperm motility and viability of post diluted semen after 210 minutes of cooling were 69.2% and 85.0%, respectively. The mean post thaw sperm motility and viability were 49.0% and 81.2%, respectively. The study concluded that the seminal attributes and results of post thaw semen quality are acceptable and indicated the feasibility of cryopreserving Khari buck semen.

Keywords: Artificial insemination; cryopreservation; goat; Khari buck; semen.

1. INTRODUCTION

Goat breeding through artificial insemination (AI) is commonly practiced worldwide. It is a very basic tool to speed up genetic improvement and increase productivity. Goat raising is a popular livestock sub-commodity of mixed farming system in Nepal. Beside food and nutritional security, it has an important role in the economy of many peoples. For the goat genetic improvement program, the government has imported some specialized exotic goat breeds and semen. Some progress has been made in the initiation of selective breeding within the local breeds and practice of AI with imported semen. Importance of AI in goats has drawn more

attention among goat farmers. However, importing semen is not a permanent solution at national level; rather, it is more important to develop and establish its own frozen semen production technique. Semen quality and its relationship with fertility are said to be a major concern in animal production, hence accurate measurement of semen fertilizing potential is of great importance (Grasa et al. 2004). The survivability of sperm cells depends on semen quality. Survivality of sperm cells is assessed through visual and microscopic evaluation of spermatozoal progressive motility and morphology. In Nepal, there is lack of scientific information on seminal attributes of buck semen, its dilution, cooling and freezing technique under management conditions of Nepal. Therefore, a study was conducted with the main objective to evaluate the seminal attributes, cooling procedures and post thaw quality of semen in Indigenous Khari Buck *(Capra hircus)* of Nepal.

2. MATERIALS AND METHOD

This study was carried out over a period of four months (March to June 2015) at Animal Breeding Division, Nepal Agricultural Research Council, Kathmandu, Nepal (27.66º N and 85.35 E; altitude of 1400 meters above sea level).

2.1 Animal and management

Two Nepalese indigenous sexually matured healthy Khari bucks aged 2.6 and 2.4 years were selected. The 2.6-year-old buck weighed 34 kg with body condition score 4 and scrotal circumference 27.4 cm. The 2.4-year-old buck weighed 36 kg with body condition score 4.5 and scrotal circumference 25.7 cm. The bucks were managed under semi-intensive system. They were allowed for natural grazing during daytime and fed with concentrate at the rate of 200 gm $head^{-1}$ day^{-1}. They were vaccinated against foot and mouth disease (FMD), *Peste des Petits Ruminants* (PPR) and dewormed with Ivermectin twice yearly.

2.2 Reagent and stain preparation

The reagents and stains were prepared as described by Jha et al. (2013). Buffered formol saline was prepared by dissolving 6.2 gm disodium hydrogen phosphate, 2.5 gm potassium dihydrogen phosphate, 5.4 gm sodium chloride and 175 ml concentrated formaldehyde in 1000 ml of distilled water. William's stain was prepared as Stock solution-I by dissolving 10 gm of basic fuchsin in 100 ml of 95% alcohol. Stock solution-II was prepared by dissolving a saturated solution of bluish eosin in 95% alcohol. Stock solution-III was prepared by mixing 10 ml of stock solution-I with 170 ml of 5% phenol solution. The final working solution contained 25 ml of stock solution-II and 50 ml of stock solution-III. The stain was left for at least two weeks for stabilization and maturation. Eosin-Nigrosin stain was prepared by dissolving 10 gm Nigrosin, 1.7 gm Eosin and 2.9 gm sodium citrate in 100 ml of distilled water. All these reagents and stains were filtered before use.

2.3 Extender preparation

The Egg Yolk Tris Citrate extender was used for semen dilution as per Ramukhithi et al. (2011). Egg Yolk Citrate Tris extender (fraction Part A and B) was prepared a day before semen collection, using the following ingredients: 1.22 gm Tris, 0.68 gm citric acid monohydrate, 0.5 gm monohydrate glucose, 7 ml glycerol (only in fraction Part B) and double distilled water (40 ml in fraction Part A and 33 ml in fraction Part B). After proper mixing, both fraction Part A and B were autoclaved, cooled to room temperature and stored at 5°C. In the morning, on the day of semen collection, 10 ml fresh hen's yolk, 50000 IU Penicillin G and 0.05 gm Streptomycin sulfate were mixed in each fraction (Part A and B) and maintained at 35°C.

2.4 Semen collection and evaluation

Artificial vagina (AV) was used to collect semen as described by Mishra et al. (2010). Bucks were trained at homosexual mount. The prepuce was wiped clean with normal saline solution and dried to reduce contamination before semen collection. Semen was collected after allowing two successive false mounts and transferred into water bath (35°C) for evaluation. Semen was evaluated according to Jha et al. (2013). Volume was recorded and color was scored visually into 1-4 grades. Sperm mass activity was estimated by placing 5µl of fresh semen on a pre-warmed (35 °C) glass slide and observed under microscope (40×), without using a cover slip. The mass activity was scored into 1-4 grades. Sperm motility was estimated by placing 5µl of fresh semen on a pre-warmed (35°C) glass slide and observed under microscope (100×), using a cover slip. Sperm concentration (10^6 ml^{-1}) was determined by using hemocytometer technique. The acrosome, mid-piece and tail abnormalities were examined by wet mount technique. A drop (10µl) of diluted fixed semen with buffered formal saline (1:100) was placed on clean glass slide with a cover slip and examined under microscope (1000×). Sperm head morphology was examined by Williams staining technique. A thin smear of fresh semen (2-4 µl) was prepared, air dried, treated with absolute alcohol for 4 minutes and air dried again. Smear was treated with 2% chlormines solution for two minutes, washed in distilled water, followed by rinsing in 95% alcohol and finally stained with carbol fuschsin for eight

minutes. Slide was washed in running tap water, dried off and examined under light microscope (1000×). Sperm viability was estimated by using eosin-nigrosin stain. A thin semen smear was prepared from one large drop (10 µl) of eosin-nigrosin stain and one small drop (2 µl) of fresh semen and examined under light microscope (400×). Live sperm cells appeared white, unstained against the purple background of nigrosin, whereas dead and damaged sperm cells appeared as pink. At least 200 spermatozoa were examined in each smear.

2.5 Semen dilution and cooling

Semen dilution and cooling was performed as described by Naing et al. (2011). Only ejaculations having ≥0.5ml semen volume, ≥80% sperm motility, ≥90% sperm viability and $\geq 2500\times10^6$ ml^{-1} sperm concentration were considered for semen dilution. Semen dilution was performed by two step dilution technique to obtain a final concentration of 100×10^6 per 0.25 ml French mini semen straw. Soon after evaluation, calculated volume of fraction Part A was mixed with semen. Diluted semen with fraction Part A and calculated volume of fraction Part B were transferred for cooling. After 90 minutes, the calculated volume of fraction Part B was mixed. The final diluted semen suspension was loaded into 0.25 ml semen straws with the help of micropipette and sealed with poly vinyl chloride (PVC) powder. Sealed straws were left further for 120 minutes for glycerol equilibration at 5°C. Semen samples were evaluated for sperm motility and viability after 90 minutes and 210 minutes of cooling.

2.6 Freezing and thawing of spermatozoa

Freezing of semen straw was done in Styrofoam box as described by Ramukhithi et al. (2011). Following glycerol equilibration, the semen straws were placed horizontally on stainless steel wire net and transferred onto the metal stand in Styrofoam box containing liquid nitrogen. The height of the metal stand was adjusted to 4.5 cm above the level of liquid nitrogen. The semen straws were exposed to liquid nitrogen vapor for 12 minutes with box top closed. Straws were plunged into liquid nitrogen. The straws were collected into goblets and transferred into Cryocan for storage. The post thaw sperm motility and viability were examined. Thawing procedure was carried out in a water bath at 37°C for 20 seconds. Data were analyzed statistically for mean and standard deviation. Data analysis was carried out with the statistical software SPSS version 20.

3. RESULTS

3.1 Seminal attributes of fresh semen

A total of 32 ejaculates (16 ejaculates from each Khari buck) were evaluated for seminal attributes. The results are presented in Table 1.

3.2 Post dilution semen quality

A total of 12 ejaculates (six ejaculates from each Khari buck) were used. The results of post-dilution motility and viability of sperm cells during cooling at 5°C are presented in Table 2. After 90 minutes post dilution, the mean sperm motility and viability were 78.8±4.8% and 88.1±2.6%, respectively. After

Table 1: Seminal attributes of fresh semen of Nepalese indigenous Khari bucks (Mean±SD)

Buck ID	Volume (ml)	Color (1-4 grade)	Mass activity (1-4 grade)	Motility (%)	Viability (%)	Concentration ($\times10^6$ ml^{-1})	Abnormal acrosome, mid-piece & tail (%)	Abnormal head (%)
Khari 1 (n=16)	0.8±0.3	3.2±0.4	3.7±0.5	83±4.7	95±2.6	2576±469	10.3±1.6	4.6±1.7
Khari 2 (n=16)	0.6±0.3	3.1±0.3	3.9±0.3	78±5.4	94±1.2	2618±347	11.1±1.9	5.4±1.6
Pooled (n=32)	0.7±0.3	3.1±0.3	3.8±0.4	81±5.6	95±2.0	2597±407	10.7±1.8	5±1.7

1= Watery; 2= Yellowish white; 3= Milky white; 4= Creamy white. Sperm mass activity (scored into 1- 4 grades): 1= No mass activity; 2= Slow wave motion without forming any waves; 3= Rapid wave motion with formation of eddies at the end of waves; 4= Very rapid wave motion with distinct with distinct eddies.

Table 2: Post-dilution motility and viability of sperm cells at 5°C (Mean±SD)

	Semen + fraction Part A (after 90 minutes)		Semen + fraction Part A + fraction Part B (after 210 minutes)	
Buck ID	Motility (%)	Viability (%)	Motility (%)	Viability (%)
Khari 1 (n=6)	77.5±2.7	89.0±3.0	73.3±4.1	85.5±1.8
Khari 2 (n=6)	70.0±3.2	87.2±2.0	65.0±4.5	84.5±1.5
Pooled (n=12)	73.8±4.8	88.1±2.6	69.2±6.0	85.0±1.7

210 minutes post dilution, the mean sperm motility and viability were 69.2±6.0% and 85.0±1.7%, respectively. The motility and viability percent decreased slightly with the advancement of cooling time.

3.3 Post thaw semen quality

The results of post-thawed motility and viability of sperm cells are presented in Table 3. The mean values of post thawed motility and viability of sperm cells were 49.0±4.6 and 82.7±2.3%, respectively.

Table 3: Post-thawed sperm motility and viability of sperm cells (Mean±SD)

Buck ID	Motility (%)	Viability (%)
Khari 1	52.0±2.7	84.2±1.5
Khari 2	46.0±4.2	81.2±1.9
Pooled	49.0±4.6	82.7±2.3

4. DISCUSSION

Desired fertility is the main goal of any breeding program. Adequate sperm functional like normal morphology, motility and ability to undergo the events of capacitation are necessary for successful pregnancy.

4.1 Seminal attributes of fresh semen

In this study, the semen volume of Khari is in agreement with Khandoker et al. (2006) and Ramukhithi et al. (2011) who reported 0.7±0.08 to 1.57±0.35 ml in South African indigenous buck and 0.43±0.03 to 0.45±0.22 ml in Black Bengal buck. In contrast, Webb et al. (2004) and Choe et al. (2006) reported semen volume 1.57±0.35 to 1.77±0.3 ml in different buck breeds. The acceptable semen volume for buck ranges from 0.5 to 2.0 ml (Al- Ghalban et al. 2004; David et al. 2007). The semen color of Khari was consistently milky white (ranged 3-4). The normal semen color of buck is yellowish-white. The color pattern of the neat semen is species specific, dependent on sperm concentration and presence of pigmented proteins and carotenoids in the seminal plasma (Hafez 1987). The sperm mass activity of Khari buck recorded rapid to very rapid wave motion with distinct eddies formation. Almost similar range was observed by Ferdinand et al. (2002) and Shamsuddin et al. (2002). The sperm motility of Khari is similar to that of Shamsuddin et al. (2002) who observed sperm motility 76.0±1.45 to 78.00±1.11% and 72.9±6.7 to 80.3±3.0% in fresh buck semen. The acceptable sperm motility of fresh buck semen is above 70% (Nur et al. 2005). In this study, the sperm viability of Khari buck is higher than 70.0-90.0% reported for different goat breeds (Igboeli 1974). The sperm cell concentration of Khari buck was within the range of 2619.58 to 2988±9.6×106 ml^{-1} found in Black Bengal buck (Faruque et al. 2007). In contrast, Shamsuddin et al. (2002) reported higher sperm concentration 4187±1768 to 7990±2360 $\times 10^9$ ml^{-1} in Bangladeshi buck. The standard sperm concentration of a good buck is $\geq 2\times10^9$ ml^{-1} (Paulenz et al. 2005; Hidalgo et al. 2007). The abnormalities of sperm morphology of Khari buck agree with that of Ghalsasi (2004) who suggested abnormal sperm acrosome, mid-piece and tail below 15% and, abnormal sperm head below 5%, as good enough for AI. The variation in seminal attributes might be due to type of breed, age of buck, season, physiological status and genetics of buck (Webb et al. 2004; Daramola et al. 2007; David et al. 2007). The feeding regime, method of collection and frequency of collection also affect semen quality (Jimenez et al. 2005; Yamashiro et al. 2006; Sundararaman et al. 2007; Zarazaga et al. 2009).

4.2 Post dilution semen quality

In our study, the slight decrease in sperm motility of Khari buck after 90 and 210 minutes of post dilution at 5°C agrees with the findings of Shamsuddin et al. (2002) who reported sperm motility 70.00±2.89 to 73.33±1.67%. Similarly, the sperm viability of Khari buck after 90 and 210 minutes of post dilution at 5°C also agrees with the findings of Samsuddin and Chanda (1998) who found 84.4±4.0% live spermatozoa. The decrease in percentage of motile and viable sperm with advancement of preservation time at 5°C is probably due to cold shock experienced by the spermatozoa (Upreti et al. 1997). The cold shock of spermatozoa is associated with oxidative stress induced by reactive oxygen species generation (Gadea et al. 2005).

4.3 Post thaw semen quality

The motility and viability of sperm cells decreased after post thawing. The post thawed sperm motility is in agreement with the results of Ramukhithi et al. (2011) who reported sperm motility of 49.3±3.4%. Sperm motility decreases up to 30% after post freezing thawing, which is assumed to be acceptable (Kozdrowski et al. 2007). However, the bench mark of post thaw sperm motility in buck is above 50% (Biswas et al. 2002). The post thawed sperm motility obtained in this study is lower than the bench mark. The quality of frozen semen decreased after thawing. During freezing and thawing process, the metabolic activity of spermatozoa is arrested and spermatozoa undergoes several ultrastructural, biochemical and functional changes. These changes are also associated with the temperature of freezing and thawing process (Maxwell and Salamon 1993; Dorado et al. 2007).

5. CONCLUSION

The seminal attributes and results of post thaw semen quality were satisfactory and in accordance with works carried out in other countries, which indicates the feasibility of cryopreserving buck semen. For more validation, the study recommends research with large number of bucks, different types of diluents and freezing trials by removing seminal plasma, followed by pregnancy rate.

Acknowledgements

The authors wish to acknowledge the Nepal Agricultural Research Council (NARC) for financial support, and providing animal shed and laboratory facilities.

REFERENCES

Al-Ghalban AM, Tabbaa MJ, and Kridli R (2004). Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small Ruminant Research, 53: 141-149.

Biswas D, Bari FY, Shamsuddin M, Rahman MM, and Rahman MM (2002). Determination of glycerol percentages for preserving the Black Bengal buck (Capra hircus) spermatozoa for long time. Pakistan Journal of Biological Sciences, 5: 715-718.

Choe CY, Kim JG, Cho SR, Son DO, Young KK, Balasubramanian S, Choe SY, and Rho GJ (2006). Influence of seasons, extenders, slow and rapid freezing on seminal characters in Koeran Native bucks. Reproduction in Domestic Animals, 41: 55-60.

Daramola JO, Adeloye AA, Fatoba TA, and Soladoye AO (2007). Induction of puberty in West African Dwarf buck-kids with exogenous melatonin. Livestock Research for Rural Development, 19: 9.

David I, Druart X, Lagriffoul G, Manfredi E, Robert-Granie C, and Bodin L (2007). Genetic and environmental effects on semen traits in Lacaune and Manech tete rousse AI rams. Genetics Selection Evolution, 39: 405-419.

Dorado J, Rodriguez I, and Hidalgo M (2007). Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. Theriogenology, 68: 168-177.

Faruque MH, Bari FY, Siddiqui MAR, and Shamsuddin M (2007). Fertilizing capacity of buck (Capra hircus) semen frozen with different concentrations of egg yolk. Journal of the Bangladesh Agricultural University, 5: 95-104.

Ferdinand N, Thomas TT, Augustave K, Henry DF, Fernand T, and Etienne PT (2002). Effects of buck age, storage duration, storage temperature and diluent of fresh West African Dwarf buck semen. Journal of Reproduction and Infertility, 3: 58-62.

Gadea J, Garcia-Vazquez F, Matas C, Gardon JC, Canovas S, and Gumbao D (2005). Cooling and freezing of Boar spermatozoa: Supplementation of the freezing media with reduced glutathione preserves sperm function. Journal of Andrology, 2: 396-404.

Ghalsasi PM (2004). Technical training manual on artificial insemination in goats. Maharashtra Goat and Sheep Research and Development Institute, Phlatan Nimbkar Agricultural Research Institute, Phlatan, India.

Grasa P, Pe-Perez R, Baguena O, Forcada F, Abecia A, Cebrian-Perez JA, and Muino-Blanco T (2004). Ram sperm selection by a dextran/swim-up procedure increases fertilization rates following intrauterine insemination in superovulated ewes. Journal of Andrology, 25: 982-990.

Hafez ESE (1987). Reproduction in Farm Animals: 315-481. 5th edition. Lea and Febiger, Philadelphia, USA.

Hidalgo M, Rodriguez I, and Dorado J (2006). Influence of staining and sampling procedures on goat sperm morphometry using the Sperm Class Analyzer. Theriogenology, 66: 996-1003.

Igboli G (1974). A comparative study of the semen and seminal characteristics of two breeds of goats. East African Agricultural and Forestry Journal, 40:132-137.

Jha PK, Paul AK, Rahman MB, Tanjim M, Bari FY, and Alam MGS (2013). Improvement of Preservation Quality of Chilled Bull Semen Using α-tocopherol as an Antioxidant. Journal of Embryo Transfer, 28: 31-39.

Jimenez F, Puchades S, Gadea J, Vicente JS, and Viudes-de-Castro MP (2005). Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. Theriogenology, 64: 1756-1765.

Khandoker MAMY, Afroz S, Islam MR, and Husain SS (2006). Cryopreservation of buck semen. In: proceedings of the XII AAAP Animal Science Congress, Bexco, Busan, Korea: 18-22.

Kozdrowski R, Dubiel A, Bielas W, and Dzieciol M (2007). Two protocols of cryopreservation of goat semen with the use of computer-assisted semen analysis system. Acta Veterinaria Brno, 76: 601-604.

Maxwell WM and Salamon S (1993). Liquid storage of ram semen: A review. Reproduction Fertility and Development, 5: 613-638.

Mishra B, Alam MGS, Khandokar MAMY, Mazumder S, and Munsi MN (2010). Qualities

of goat semen in Tris-Citrate-Glucose extender containing glutathione. Bangladesh Veterinarian, 27: 46-55.

Naing SW, Haron AW, Khan MA, and San MM (2011). Effect of seminal plasma removal, washing solutions, and centrifugation regimes on Boer Goat semen cryopreservation. Pertanika Journal of Tropical Agricultural Science, 34: 271-279.

Nur Z, Dogan I, Gunay U, and Soylu KM (2005). Relationships between sperm membrane integrity and other semen quality characteristics of the semen of Saanen goat bucks. Bulletin of the Veterinary Institute in Pulawy, 49: 183-187.

Paulenz H, Soltun K, Adnoy T, Andersen K, and Soderquist L (2005). Effect of different extenders on sperm viability of buck semen stored at room temperature. Small Ruminant Research, 59: 89-94.

Ramukhithi FV, Nedambale TL, Sutherland B, and Lehloenya KC (2011). Cryopreservation of South African indigenous goat semen. African Journal of Biotechnology, 10: 17898-17902.

Shamsuddin M and Chanda PK (1998). Effects of diluents and dilution rates on the preserved buck semen. In: 4th Asian Symposium on Animal Biotechnology. Kamiina, Japan: 177-181

Shamsuddin M, Amiri Y, and Bhuiyan MMU (2002). Characteristics of buck semen with regard to ejaculate numbers, collection intervals, diluents and preservation periods. Reproduction in Domestic Animals, 35: 53-57.

Sundararaman MN, Kalatharan J, and Edwin MJ (2007). Attempts to achieve semen collections from incapacitated Boer bucks by electro-ejaculation. Asian Journal of Animal and Veterinary Advances, 2: 244-246.

Upreti GC, Jensen K, Oliver JE, Duganzich DM, Munday R, and Smith JF (1997). Motility of ram spermatozoa during storage in a chemically-defined diluent containing antioxidants. Animal Reproduction Science, 48: 269-278.

Webb EC, Dombo MH, and Roets M (2004). Seasonal variation in semen quality of Gorno Altai cashmere goats and South African indigenous goats. South African Society for Animal Science, 34: 240-243.

Yamashiro H, Kumamoto K, Wang H, Yamashita Y, and Terada T (2006). Effect of semen collection in extender solution on the characteristics of goat spermatozoa. Journal of Reproduction and Development, 52: 397-406.

Zarazaga LA, Guzman JL, Dominguez C, Perez MC, and Prieto R (2009). Effects of season and feeding level on reproductive activity and semen quality in Payoya buck goats. Theriogenology, 71: 1316-1325.