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Full Length Research Paper

Cryopreservation of South African indigenous goat semen

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The aim of this study was to cryopreserve and evaluate frozen-thawed semen parameters. Electro-ejaculator was used to collect semen from six bucks. Semen samples were divided into two parts. In one part, seminal plasma was removed and was not in the other one. Both semen sample parts were extended with egg-yolk Tris extender. Semen was thereafter cryopreserved in straws using liquid nitrogen. Data were analysed using Stata® V10 software. South African indigenous goats had total sperm cell motility rate of 83.1%, progressive sperm cell motility of 49.3% and non-progressive sperm cell motility of 33.9%. Moreover, acidic semen pH of 6.4 and low sperm cell concentration (663.6 × 10^6/ml) were obtained. Removal of seminal plasma and semen cryopreservation significantly decreased pH (5.8 ± 0.1 and 5.7 ± 0.1 for frozen-thawed washed and not-washed, respectively) and sperm cell motility rates of South African indigenous goats. Reduction in the sperm cell motility after freeze/thawing is still a problem and requires further research on the diluents and techniques that give protection to sperm cells during cryopreservation.

Key words: Cryopreservation, semen characteristics, indigenous goat.

INTRODUCTION

South African indigenous goats are resistant to different parasites and diseases such as blue tongue and able to survive on the poorest vegetation and are well known for being non-selective browsers (Agricultural Research Council, 2006). Majority of South African indigenous goats are kept in rural areas and they serve as a source of milk, hides and meat (Webb et al., 2003). However, this goat breed is under threat of extinction and very little information on the reproductive status is known (Webb et al., 2003). Fresh semen characteristics that were previously obtained from other South African indigenous goats (Boer) were; semen volume of more than 1.5 ml (Tuli and Holtz, 1995; Sundararaman et al., 2007), sperm cell motility of 72% (Tuli and Holtz, 1995), sperm cell concentration of greater than 3 billion sperm cells (Motlomelo et al., 2002). For frozen-thawed semen, progressive sperm cell motility of 54% was reported (Tuli and Holtz, 1995). However, there is no study done yet on the cryopreservation of South African indigenous goat semen.

Cryopreservation of goat semen is indicated for several reasons (Martinez et al., 2007). The cryopreserved sperm cells can be stored and used after a long period of time. Cryopreservation of goat sperm cells also extends the reproductive life of a buck after his own life (Rahman et al., 2008). Frozen-thawed goat semen can be utilised for artificial insemination to enhance improvement of livestock as breeders mostly use genetically superior bucks (Martinez et al., 2007). Goat semen has been cryopreserved, using Tris-based extender, which contains a safe margin of egg yolk to avoid coagulation and provide sperm cells with nutrients like protein (Ritar et al., 1990). However, the sperm cell motility rates following cryopreservation have been usually lower than when fresh semen is used. For example, goat fresh sperm cell

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motility generally ranges from 80 to 90% (Gacitua and Arav, 2005). However, Purdy (2006) reported no or low recovery rates (0 to 18%) of motile sperm cells post-thaw in goats. On the other hand, the post/freezing sperm cell motility ranges from 23 to 65 % between different goat breeds (Tuli and Holtz, 1995; Gacitua and Arav, 2005). These clearly show that sperm cell motility following freezing is relatively low, which also lead to low conception rate following artificial insemination (Lopez-Sebastian et al., 2007; Houdeau et al., 2008). Therefore, an increase in the recovery of good quality sperm cells following semen cryopreservation is necessary to achieve reasonable fertility rates (Gacitua and Arav, 2005). The objective of this study was to cryopreserve and evaluate South African indigenous goat frozen-thawed semen parameters.

MATERIALS AND METHODS

The trial was conducted at the Agricultural Research Council (Irene), South Africa. Six South African indigenous bucks aged 2 to 8 years were used for semen collection. All the experimental goats were grazing on natural pasture (kikuyu). Water was provided *ad libitum* throughout the experimental period. Semen samples were collected once a week for 10 weeks during the breeding (autumn) season by means of an electro-ejaculator (Ramsem, South Africa). One ejaculate was collected from each buck during a collection session. Semen was collected in pre-warmed (37°C) 15 ml graduated tubes (Cell Star® tubes, Greiner Bio-One GmbH, Germany) (Zarazaga et al., 2009). Semen samples were kept approximately for 10 min in a thermoflask at 37°C for transportation to the laboratory for evaluation.

Semen samples were evaluated for volume, pH, sperm cell motility and concentration. The volume of the ejaculates was measured by reading the measurements on the 15 ml graduated tube. Semen volume was recorded in millilitres. Semen pH was determined by using a pH meter [(HANNA Instruments®, South Africa (Pty) (Ltd)] while sperm cell motility was determined using a Sperm Class Analyser® (Microptic S.L, Barcelona). The sperm cell motility was recorded in percentages. The sperm cell concentration was determined by using a Spermacue® photometer (Minitube®, South Africa). The sperm cell concentration was recorded in millions per millilitre.

After semen evaluation, fresh semen from each buck was divided into halves. One part was mixed at a ratio of 1:1.5 with prepared synthetic oviductial fluid (25°C). Then the semen samples which have been mixed with synthetic oviductial fluid [(Sigma-Aldrich, South Africa) (Pty) (Ltd)] were centrifuged at 1500 g for 10 min. Then seminal plasma was then removed using plastic pipette (Minitube®, South Africa), leaving only the sperm cell pellet.

A day before semen collection, egg yolk-Tris extender (Fraction A and B), used to dilute semen, was prepared using the following: Tris (1.221 g), citric acid (0.68 g), monohydrate glucose (0.5 g), gentamycin sulphate (50 mg), glycerol (7 ml) (only in Fraction B) [(Sigma-Aldrich, South Africa) (Pty) (Ltd)], chicken egg yolk (10 ml) and sterile water (40 and 33 ml for Fraction A and B, respectively) [(Transfarm, South Africa) (Pty) (Ltd)]. All these components were added together in a 50 ml graduated tube (Cell Star® tubes, Greiner Bio-One GmbH, Germany) and secured with a cap, then stored at 5°C. On day of semen collection, the prepared extender (Fraction A) was warmed in the water bath at 37°C.

Both semen parts (with and without seminal plasma) were extended 1:1 with Fraction A of egg yolk-Tris extender. The extended semen samples were equilibrated for an hour at 25°C.

Then the semen samples were extended 1:1 with Fraction B of egg yolk-Tris extender and were equilibrated at 5°C for 2 h. During the last 10 min of 2 h semen equilibration, all the extended semen samples were loaded into 0.25 ml plastic straws (Minitube®, South Africa) (Purdy, 2006). After filling, the semen straws were sealed by using dry polyvinyl powder (Minitube®, South Africa) and were placed on a semen freezing holding racks.

For freezing, the Styrofoam container was filled with liquid nitrogen (Afrox, South Africa). The rack carrying semen straws was suspended for 10 min above liquid nitrogen, keeping a gap of 5 cm between the surface of liquid nitrogen and the semen straws. After 10 min, the straws containing semen were submerged into liquid nitrogen. Then the semen straws from each buck were grouped and loaded into a liquid nitrogen canister and stored in at -196°C for approximately 3 months. Semen straws were thawed, by dipping them into a water bath at 37°C for 20 s before evaluation. Five semen straws per buck per treatment (washed and not-washed) were thawed for evaluating sperm cell motility and pH. Data was expressed as mean ± S.E and the analysis was performed at 95% confidence limit, using Stata® V10 software (StataCorp. 2009).

RESULTS

The fresh semen characteristics of South African indigenous goats are shown in Table 1. Frozen-thawed (not-washed and washed) semen characteristics of South African indigenous goats are shown in Table 2. Seminal plasma removal and semen freezing resulted to significantly ($p < 0.05$) lower pH (acidic), lower total, progressive and non-progressive sperm cell motility. These eventually increased immotile sperm cell. In frozen-thawed semen sample, the percentage of immotile sperm cells was higher for washed than not-washed semen sample. However, both washed and not-washed semen samples still had higher percentage of immotile sperm cells.

DISCUSSION

In this study, the semen volume of 0.7 ± 0.08 ml for South African indigenous goat was lower compared to the semen volume of 1.57 ± 0.35 ml (Dombo, 2002) and 1.77 ± 0.3 ml (Webb et al., 2004), obtained during the natural breeding season using the same method of semen collection and similar goat breed. The differences between the present and previous studies conducted using similar breed might be due to the age of bucks used (Daramola et al., 2007). For this study, young to old bucks were used (2 to 8 years), whereas in the previous study, mature bucks were used (4 to 5 years). Lack of mineral supplementation in the experimental bucks of this current study might have also contributed to low semen volume compared to the previous studies using similar breed. According to Almeida et al. (2007), feed and mineral supplementation in winter is reported to have beneficial effect on the testicular development and semen quality and quantity in bucks. Furthermore, semen volume was also suggested to be affected by physiological status and genetic effects of the bucks (Webb et al., 2004; David et al., 2007). Moreover, this

Table 1. Fresh semen characteristics (mean ± S.E) of South African indigenous goats.

Semen characteristics	Mean ± S.E
Volume (ml)	0.7 ± 0.08
Concentration (10^6/ml)	663.6 ± 33.3
pH	6.4 ± 0.2
Progression (%)	
TM	83.1 ± 3.3
PM	49.3 ± 4.3
NPM	33.8 ± 4.7
Immotile	16.9 ± 3.3

TM = Total motility, PM = Progressive motility and NPM = Non-progressive motility

Table 2. Effect of cryopreservation on semen quality (mean ± S.E) of South African indigenous goats.

Semen	pH	Progression (%)			
		TM	PM	NPM	Immotile
Fresh	6.3 ± 0.1^a	83.1 ± 2.7^a	49.3 ± 3.4^a	33.8 ± 3.3^a	16.9 ± 2.7^a
F-T not-washed	5.7 ± 0.1^b	28.2 ± 4.3^b	13.2 ± 2.3^b	15 ± 2.7^b	71.8 ± 4.2^b
F-T washed	5.8 ± 0.1^b	13.8 ± 4.1^c	5.5 ± 1.8^c	8.3 ± 2.5^c	86.2 ± 4.1^c
P – value	0.048	0.029	0.035	0.048	0.026

a, b, c Values with different superscripts within same column differ significantly ($p < 0.05$).
F-T = Frozen-thawed thawed, TM = total motility, PM = progressive motility and NPM = non-progressive motility.

semen volume obtained in this current study was also lower compared to the semen volume of 2.1 ± 1.0 ml obtained from other breeds in other countries (Choe et al., 2006). For the latter case, breed might have led to the differences. It has been reported that some breeds produce higher semen volume (Webb et al., 2004). Though the semen volume was lower comparable to previous research, it was still within acceptable semen volume for goats (0.5 to 2.0 ml) as suggested by Al-Ghalban et al. (2004) and David et al. (2007).

The sperm cell concentration of 663.6 × 10^6/ml was significantly higher compared to the previous sperm cell concentration of 116.7 × 10^6/ml reported by Dombo (2002) using similar South African indigenous goat breed. The difference between this current study and the previous study might be due to different instruments that were used to evaluate sperm cell concentration. In previous study, haemocytometer was used whereas in this current study, Spermacue® photometer was used. However, the sperm cell concentration of this current study was lower compared to sperm concentration of 4762 × 10^6/ml reported by Hidalgo et al. (2006) using Florida goats. The method of semen collection might have also affected the results (Jimenez et al., 2005), as in Hidalgo et al. (2006) experiment, artificial vagina method of semen collection was used and in this current study electro-ejaculator method of semen collection was used. Furthermore, the results of this current study were not meeting the standard sperm cell concentration of ≥ 2 × 10^9 sperm cells/ml that a good buck must produce (Paulenz et al., 2005; Hidalgo et al., 2007). In general, in terms of sperm cell concentration the results of this current study were poor, but higher than previous reports using the same breed. The sperm cell motility of South African indigenous goat was acceptable and higher (83%) as it was above 70% compared to the sperm cell motility of 62.5% reported by Nur et al. (2005) when Saanen goats were used. Acceptable fresh sperm cell motility obtained in this study might be due to the reason that the semen samples were collected during the natural breeding season (autumn). This was also in agreement with Gacitua and Arav (2005) who indicated that goat sperm cell motility is high during the breeding season than during the non-breeding season. There are several factors which might have caused differences between this current study and previous studies in terms of sperm concentration, this include breed used, nutrition, time of the year and the age of the goats (Jimenez et al., 2005; Bester, 2006; Daramola et al., 2006; Yamashiro et al., 2006; Sundararaman et al., 2007; Zarazaga et al., 2009).

South African indigenous goat semen had more acidic semen pH when compared to the semen pH of 7.0 and

7.2 which was documented to be most favourable to the sperm cell motility rate in other goat breeds (Molinia et al., 1994; Purdy, 2006). The causes of acidic semen pH are unknown, but it was assumed that electro-ejaculator during semen collection had a negative effects in South African indigenous goats. Electro-ejaculator method is reported to stimulate a buck to release acidic urine which contaminate semen and reduce the semen pH (Jimenez et al., 2008; Moreno et al., 2009).

In this study, seminal plasma removal with a physiological solution seems to decrease the percentage of motile sperm cells. Similar results were recorded by Azeredo et al. (2001) who reported that parts containing seminal plasma had high sperm cell motility than semen without seminal plasma. However, contradictory results to this current study were reported, indicating that seminal plasma removal with a physiological solution increased the percentage of motile sperm cells before and after freezing in goats (Leboeuf et al., 2000; Gacitua and Arav, 2005). Therefore, more research on appropriate solutions or ingredients is recommended

The results of this current study also showed that cryopreservation decreases sperm cell motility. These results are in agreement with Kozdrowski et al. (2007) who obtain less than 30% sperm cell motility. Moreover, the frozen-thawed sperm cell motility obtained in this study was lower than the sperm cell motility bench mark of 50% recommended by Biswas et al. (2002) in goat. A decrease in cryopreserved sperm cell motility might be due to ultrastructural, biochemical and functional changes of cryopreserved sperm cells. These changes are caused by freezing temperature and thawing process (Dorado et al., 2007; Uysal and Bucak, 2009). Moreover, a decrease in post-thawing motility of sperm cells was suggested to be due to more acidic semen pH (Molinia et al., 1994). This is because the South African indigenous goat semen pH continued to be more acidic following cryopreservation. Acidic semen pH usually results from sperm cell apoptosis that occur during cryopreservation due to toxic substances secreted during death of the other sperm cells (Bester, 2006; Uysal and Bucak, 2009).

Different methods for processing (semen evaluation and dilution) and freezing of semen using vapour and programmable freezers have been developed to reduce cryogenic injuries to sperm cells (Dorado et al., 2007). Nevertheless, the results of this study indicated that some of the methods developed to reduce cryogenic injuries to sperm cells are not helping as the results obtained are not satisfactory. Following unsatisfactory results of this current study, it was believed that the ability to freeze goat semen is still a challenge as it involves balancing of many variables (Rahman et al., 2008). Therefore, some modification or adjustment must be made on the freezing method, equilibration and cooling rate, cryoprotective diluents, dilution and thawing method. In conclusion, the semen volume of South African indigenous goat fresh semen was 0.7 ml and its pH (6.4 ± 0.2) was acidic. The sperm cell motility of South African indigenous goat was acceptable. In terms of sperm cell concentration, it was below the required standard concentration that the good buck must produce. This study also demonstrated that cryopreservation of South African indigenous goat semen decreased semen pH and sperm cell motility, whether seminal plasma was removed or not. Reduction in the sperm cell motility after freeze/thawing is still a problem and requires further research on the diluents and techniques that give protection to sperm cells during cryopreservation.

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REFERENCES

Agricultural Research Council [Internet] (2006). The Indigenous goat breeds of SA; [Cited 2008 July 18]. Available from: http://www.arc.agric.za/home.asp?pid=2701.

Al-Ghalban AM, Tabbaa MJ, Kridli RT (2004). Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small Rumin. Res. 53: 141 – 149.

Almeida AM, Schwalbach LMJ, Cardoso LA, Greyling JPC (2007). Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. Small Rumin. Res. 73: 216-220.

Azeredo GA, Esper CR, Resende KT (2001). Evaluation of plasma membrane integrity of frozen-thawed goat spermatozoa with or without seminal plasma. Small Ruminant Res. 41: 257-263.

Bester N (2006). Effect of different dietary energy levels on productive and reproductive traits in Dorper rams. MSc dissertation, Bloemfontein, University Free State.

Biswas D, Bari FY, Shamsuddin M, Rahman MM (2002). Determination of Glycerol percentages for preserving the Black Bengal buck (*Capra hircus*) spermatozoa for long time. Pak. J. Biol. Sci. 5(6): 715-718.

Choe CY, Kim JG, Cho SR, Son DS, Kim YK, Balasubramanian S, Choe SY, Rho GJ (2006). Influence of seasons, extenders, slow and rapid freezing on seminal characters in Korean native bucks. Rep. Domest. Anim. 41: 55–60.

Daramola JO, Adeloye AA, Fayeye TR, Fatoba TA, Soladoye AO (2006). Influence of photoperiods with or without melatonin on spermiograms in West African Dwarf bucks. World J. Zool. 1(2): 86-90.

Daramola JO, Adeloye AA, Fatoba TA, Soladoye AO (2007). Induction of puberty in West African Dwarf buck-kids with exogenous melatonin. Livest. Res. Rural Dev. 19(9).

David I, Druart X, Lagriffoul G, Manfredi E, Christele R, Bodin L (2007). Genetic and environmental effects on semen traits in Lacaune and Manech tête rousse AI rams. Genet. Sel. Evol. 39: 405-419.

Dombo MH (2002). Seasonal effect on semen quality of Gorno Altai and South African indigenous goats. MSc dissertation, Pretoria, University of Pretoria.

Dorado J, Rodriguez I, 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.

Gacitua H, Arav A (2005). Successful pregnancies with directional freezing of large volume buck semen. Theriogenology, 63: 931-938.

Hidalgo M, Rodriguez I, Dorado J (2006). Influence of staining and

sampling procedures on goat sperm morphometry using the sperm class analyzer Theriogenology, 66: 996 – 1003.
Houdeau E, Furstoss V, Forgerit Y, Bonne JL, Leboeuf B (2008). Short duration insemination with frozen semen increases fertility rate in nulliparous dairy goats. Anim. 2(10): 1496–1500.
Jimenez F, Puchades S, Gadea J, Vicente JS, Viudes-de-Castro MP (2005). Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. Theriogenology, 64: 1756–1765.
Jimenez F, Vicente JS, Viudes-de-Castro MP (2008). Seminal plasma composition from ejaculates collected by artificial vagina and electro-ejaculation in Guirra ram. Rep. Domest. Anim. 43: 403-408.
Kozdrowski R, Dubiel A, Bielas W, Dzięcioł M (2007). Two protocols of cryopreservation of goat semen with the use of computer-assisted semen analysis system. Acta Vet. Scand. 76: 601-604.
Leboeuf B, Restall B, Salamon S (2000). Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci. 62: 113-141.
Lopez-Sebastioan A, Gonzalez-Blues A, Carrizosa JA, Urrutia B, Diaz-Delfa C, Santiago-moreno J, Gomez-Brunet A (2007). New oestrus synchronisation and artificial insemination protocol for goats based on male exposure, progesterone and cloprostenol during the non-breeding season. Theriogenology, 68: 1081-1087.
Martinez RRD, Hernandez J, Reyna L. Santos R (2007). Intrauterine insemination with frozen-thawed semen in Creole goats, synchronized in oestrous during the non-breeding season. Res. J. Anim. Sci. 1(3): 76-80.
Molinia FC, Evans G, Quintana C, Maxwell WMC (1994). Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim. Reprod. Sci. 36: 113-122.
Moreno JS, Coloma MA, Dorado J, Pastor AP, Gomez-Guillamon F, Salas-Vega R, Gomez-Brunet A, Sebastian LA (2009). Cryopreservation of Spanish ibex (Capra pyrenaica) sperm obtained by electro ejaculation outside the rutting season. Theriogenology, 71: 1253-1260.
Motlomelo KC, Greyling JPC, Schwalbach LMJ (2002). Synchronisation of oestrous in goats: the use of different progestagen treatments. Small Rumin. Res. 45: 45-49.
Nur Z, Dogan I, Gunay U, Soylu MK (2005). Relationships between sperm membrane integrity and other semen quality characteristics of the semen of Saanen goat bucks. B. Vet. Pulawy. 49: 183-187.
Paulenz H, Soltun K, Ådnøy T, Berg KA, Söderquist L (2005). Effect of different extenders on sperm viability of buck semen stored at room temperature. Small Rumin. Res. 59: 89-94.
Purdy PH (2006). A review on goat sperm cryopreservation. Small Rumin. Res. 63: 215-225.
Rahman ANMA, Abdullah RB, Khadijah WEW (2008). A review of reproductive biotechnologies and their application in goat. Biotechnology, 7(2): 371-384.
Ritar AJ, Ball PD, O'May PJ (1990). Examination of methods for the deep freezing of goat semen. Reprod. Fertil. Dev. 2: 27-34.
Sundararaman MN, Kalatharan J, Edwin MJ (2007). Attempts to achieve semen collections from incapacited Boer bucks by electro-ejaculation. Asian. J. Anim. Vet. Adv. 2(4): 244-246.
Tuli RK, Holtz W (1995). Effect of season on the freezability of Boer goat semen in the Northern temperate zone. Theriogenology, 43: 1359–1363.
Uysal O, Bucak MN (2009). The role of different trehalose concentrations and cooling rates in freezing of ram semen. Ankara Univ. Vet. Fak. Derg. 56: 99–103.
Webb EC, Mamabolo MJ, du Preez ER, Morris SD [Internet] (2003). Reproductive status of goats in communal systems in South Africa; [Cited 2011 July 18]. Available from: http://www.up.ac.za/asservices/ais/vet/sec41.
Webb EC, Dombo MH, Roets M (2004). Seasonal variation in semen quality of Gorno Altai cashmere goats and South African indigenous goats. S. Afr. J. Anim. Sci. 34: 240–243.
Yamashiro H, Kumamoto K, Wang H, Yamashita Y, Terada T (2006). Effect of semen collection in extender solution on the characteristics of goat spermatozoa. J. Reprod Dev. 52 (3): 397-406.
Zarazaga LA, Guzman JL, Dominguez C, Perez MC, Prieto R (2009). Effects of season and feeding level on reproductive activity and semen quality in Payoya buck goats. Theriogenology, 71: 1316-1325.

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