

1-1-2013

Effect of extenders, postdilution intervals, and seasons on semen quality in dairy goats

MUHAMMAD SUBHAN QURESHI

DAULAT KHAN

ANILA MUSHTAQ

SHOAIB SULTAN AFRIDI

Follow this and additional works at: <https://journals.tubitak.gov.tr/veterinary>



Part of the [Animal Sciences Commons](#), and the [Veterinary Medicine Commons](#)

Recommended Citation

QURESHI, MUHAMMAD SUBHAN; KHAN, DAULAT; MUSHTAQ, ANILA; and AFRIDI, SHOAIB SULTAN (2013) "Effect of extenders, postdilution intervals, and seasons on semen quality in dairy goats," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 37: No. 2, Article 4. <https://doi.org/10.3906/vet-1110-24>
Available at: <https://journals.tubitak.gov.tr/veterinary/vol37/iss2/4>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Veterinary & Animal Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Effect of extenders, postdilution intervals, and seasons on semen quality in dairy goats

Muhammad Subhan QURESHI*, Daulat KHAN, Anila MUSHTAQ, Shoaib Sultan AFRIDI
Faculty of Animal Husbandry and Veterinary Science, KP Agricultural University, Peshawar 25120, Pakistan

Received: 16.10.2011 • Accepted: 12.06.2012 • Published Online: 15.03.2013 • Printed: 15.04.2013

Abstract: The breeding of female dairy goats with good quality sires has been a problem in rural areas and artificial insemination (AI) provides an answer to this issue. To develop an AI model, a total of 9 bucks were purchased from a local market. Semen was collected in an artificial vagina, the libido was noted, and the semen was evaluated for its physical characteristics. The libido, semen volume, mass motility, individual motility, and sperm concentration were 2.94 ± 0.25 (scale of 1–3), 0.79 ± 0.59 mL, 4.34 ± 0.93 (scale of 1–5), $55.01 \pm 32.59\%$, and 1027 ± 406 million spermatozoa/mL, respectively. The buck affected various quality parameters ($P < 0.05$) except individual motility, which may be confounded by the time interval. The libido declined during June and the mass motility declined from November to January. The individual motility remained higher during March and July to October. The volume was the highest during January and the sperm concentrations were the highest during November. No difference was found in the sperm motility up to 2 h after dilution. Between 6 and 192 h, the sperm motility decreased significantly from 56.43% to 10.71%. The extender containing 20% egg yolk and 7% glycerol (control) maintained sperm motility for more than 192 h (72.33%). The time of collection correlated with the semen volume significantly ($r = -0.237$, $P = 0.000$). The semen volume correlated positively with the libido and negatively with the mass motility and concentration. The concentration correlated positively with the time of collection and the mass motility. The individual and mass motility correlated positively with each other.

Key words: Semen, goats, extender, motility, dairy, longevity

1. Introduction

In rural areas, farmers usually have a small number of goats and also have limited access to a high quality sire or even a sire at all, due to which there is no breeding of the goat coming into heat through natural service. Artificial insemination (AI) is the solution to this problem at the field level. Many countries have already started AI in goats. For example, in Poland, AI in goats was started during 1987 and continued for only 3 years, and then the practice was restarted with some technical modifications, which resulted in a great increase in the goat population and genetic potential (1). A study was initiated for establishing an AI service in dairy goats at Peshawar (2). Tris, glucose, citrate, and yolk extender gave sufficient protection to the spermatozoa for up to 192 h after collection. The semen was evaluated in vivo through insemination to live goats and was pursued for the fate of the insemination. The conception rate after insemination was 75%.

The storage of frozen goat semen has been reported to cause ultrastructural, biochemical, and functional damage to the spermatozoa, resulting in a reduction of motility, viability, and fertility and impaired transport (3). A pregnancy rate of about 61% or 64% was obtained using

cryopreserved or fresh semen in does in which estrus was synchronized by means of various hormones.

For the preservation of buck semen, different extenders have been used, some of which are skim cow milk, sodium citrate glucose yolk, lactose yolk, saccharose EDTA, calcium nitrite yolk, raffinose yolk, Spermasol (Witco Chemical Corp., Oakland, NJ, USA), and Tris yolk. The egg yolk-coagulating enzyme, phospholipase, which is secreted into the seminal plasma through the bulbourethral glands, hydrolyses the egg yolk lecithin into fatty acids, with the result that lysolecithin may cause a determinable effect on buck semen (3). The glycerol-containing extender exhibited a negative effect on the semen quality of bucks, reflected by a reduction in the quality parameters in a higher number of bucks (4). The study suggested a harmful effect of glycerol on the membrane integrity of human and sheep spermatozoa when it was used as a cryoprotectant.

Most of the research work has been conducted in western countries on semen extenders for goats. Considering the subtropical conditions of Peshawar and the pivotal role of goats in the local economy, the present study was designed to investigate the effect of various extenders and postcollection intervals on the semen quality in goats.

* Correspondence: drmsqureshi@aup.edu.pk

2. Materials and methods

2.1. Animal selection and management

A total of 9 goats were purchased from a local market, consisting of 5 females and 4 males. The goats were accommodated in a shed providing a partition between the males and females. The animals were grazed in the nearby adjoining areas on a daily basis, providing an opportunity for exercise. Green fodder was provided ad libitum and concentrates were fed according to requirements. Free access to drinking water was provided. The body condition score was determined as described by Hervieu et al. (5), ranging from 0 to 5. In addition to the subcutaneous fat per se, large amounts of substernal fats also accumulate in the sternal region and can be felt on palpation, and so they are used to assign a body condition score.

Data were collected twice a week on a periodical basis. On the day of semen collection, the extenders and all of the glassware were brought to room temperature and the artificial vaginas were maintained at 42 °C. The bucks were introduced to a waiting lawn, one by one, and presented individually to the does.

2.2. Semen collection and evaluation

Semen was collected in an artificial vagina from 0800 to 1100 hours and the volume was measured in a graduated semen collection tube. The bucks were exposed to teasers for a period of 5 min. Libido was recorded at a scale of 0 to 3, with 0 being the complete absence of sexual desire and 3 being the highest level of sexual desire giving minimal time to jump over the teaser animal. Levels 1 and 2 lie between the 2 extremes. The tube was placed in a water bath at 34 °C. A drop of semen was taken, placed on a slide, and examined for concentration proportionate to the darkness of the sample, as described by Qureshi (2), under

a microscope at 4× magnification reflecting a spermatozoa concentration of 0 to 2000 m/mL. The mass motility (reflected by wave motion) of the whole semen sample was checked and graded as 0–5. Next, the individual motility of the semen was determined after diluting the semen with an extender in a reagent bottle, keeping a spermatozoa concentration of about 20 million/mL.

The diluted semen was kept in a reagent bottle that was placed in a pot containing warm water (35 °C). The pot was placed in a refrigerator at 5 °C for liquid preservation of semen. The temperature decreased to 5 °C slowly over a period of 1 h. The individual motility of the spermatozoa was recorded as percentages for 0, 0.5, 1, 2, 6, 24, 48, 96, 144, and 192 h postdilution. A drop of the diluted semen was placed on a warm glass slide at around 38 °C, enclosed with a cover slip, and examined for sperm motility (%) at 10× and 40× magnifications.

2.3. Extenders preparation

Extenders were prepared for evaluation and the long storage of the semen under refrigeration. Table 1 gives the composition of the extenders. The ingredients were weighed on an electronic balance, put in a beaker with 73 mL of distilled water, and placed on a heated magnetic stirrer overnight. Next, the beaker was placed in hot water (90 °C) for 15 min. In the case of egg yolk, it was placed into a 100-mL beaker, added to glycerol, and transferred to a 100-mL conical flask, and then 2 g of Penbiotic was added to this solution. The pH was adjusted to 6.8. The flask was labeled and kept in a refrigerator for semen dilution.

2.4. Statistical analysis

The semen quality parameters are reported as the means for various seasons, postdilution intervals, and extenders.

Table 1. Compositions of the extenders used for the processing of the goat semen.

Ingredient	Extender										
	1	2	3	4	5	6	7	8	9	10	11
Lactose	6 g	5 g	-	6 g	-	-	-	-	-	-	6 g
Fructose	1 g	1.5 g	-	1 g	-	-	-	1 g	0.2 g	1 g	1 g
Glucose	-	-	-	-	1 g	1 g	1 g	-	-	-	-
Cow milk	-	-	100 mL	-	-	-	-	-	-	-	-
Buffalo milk	-	-	-	-	-	-	-	-	-	-	-
Goat milk	-	-	-	-	-	-	-	-	-	-	-
Egg yolk	20 mL	15 mL	-	-	10 mL	10 mL	10 mL	20 mL	-	-	20 mL
Tris	-	-	-	-	2.4 g	2.4 g	2.4 g	2.4 g	4 g	2.4 g	-
Citric acid	-	-	-	-	1.4 g	1.4 g	1.4 g	1.3 g	2 g	1.4 g	-
Glycerol	7 mL	5 mL	-	-	10 mL	5 mL	-	7 mL	1.4 mL	1.4 mL	7 mL
Penbiotic	2 g	2 g	2 g	2 g	1 g	1 g	1 g	2 g	2 g	2 g	2 g
D. water	73 mL	73 mL	-	100 mL	80 mL	85 mL	90 mL	73 mL	93 mL	98 mL	73 mL

Analysis of variance was used for comparing the means for the semen quality parameters as affected by the bucks, seasons, and extenders. Duncan's multiple range test was used for ranking the means across various class variables. The time of collection, libido, semen volume, mass motility, and individual motility were correlated with each other and the correlation coefficient was determined with the value of probability for each interaction. The general linear model procedure was used for analysis in SPSS (6) under the guidelines of Steel and Torrie (7).

3. Results

A total of 480 semen collections were made through an artificial vagina. The libido, semen volume, mass motility, individual motility, and sperm concentration were 2.94 ± 0.25 (scale of 1–3), 0.79 ± 0.59 mL, 4.34 ± 0.93 (scale of 1–5), $55.01 \pm 32.59\%$, and 1027 ± 406 million spermatozoa/mL, respectively (Table 2).

3.1. Effect of the donor

The buck affected various quality parameters of the semen including the libido, volume, mass motility, and sperm concentrations ($P < 0.05$), but not the individual motility. The individual motility was also recorded during various postcollection intervals; its response to the buck may be confounded by the time interval. The best values for mass motility, individual motility, and libido were shown by buck number 1 (5.00, 59.97%, 3.00), the best volume was by buck number 2 (0.88 mL), and the best sperm concentration was by buck number 3 (1124.44 million/mL).

3.2. Effect of season

The semen volume, individual motility, and sperm concentration were significantly affected by the seasons ($P < 0.05$, Table 2). Spring was found as the normal breeding season for goats in this study. A slight decline was observed in the libido during the summer and in the mass motility during the autumn. The best semen volume and individual motility were recorded during the spring and the highest concentrations were recorded during the winter.

3.3. Semen survivability

After dilution, the semen was examined for the individual sperm motility at different intervals of time (0, 0.5, 1, 2, 6, 24, 48, 96, 144, and 192 h). No statistical difference was found in the sperm motility at 0, 0.5, 1, and after 2 h (66.43, 66.43, 66.19, and 64.05, respectively). Between 6 and 192 h, the sperm motility decreased significantly from 56.43% to 10.71%. The sperm motility decrease after 3 to 6 h was 7.62%, 14.05% after 7 to 24 h, 3.57% after 25 to 48 h, 9.29% after 49 to 96 h, 6.42% after 97 to 144 h, and 2.39% after 145 to 192 h. A noticeable decline in the individual motility was observed from 7 to 24 h (14.05%) and 25 to 48 h (13.57%; Figure).

3.4. Effect of extenders

Eleven different extenders were used for the individual motility of the spermatozoa at different postdilution intervals. The type of extender significantly ($P < 0.01$) affected the individual sperm motility percentage (9, 75.67%; 10, 75.00%; 1, 72.33%; 5, 62.00%; 8, 61.00%; 2, 58.33%; 7, 51.00%; 3, 46.33%; 11, 39.67%; 4, 39.33%; and 6, 27.00%, respectively). Extenders 1, 9, and 10 maintained

Table 2. Effect of donors and seasons on the various parameters of the semen quality in dairy bucks. Differing letters in columns show significance.

Independent variable	N	Libido	Volume (mL)	Mass motility	Individual motility (%)	Sperm concentrations (millions/mL)
Overall mean	478	2.94	0.79	4.34	55.01	1027
Donors (buck number)						
1	77	3.00 ^a	0.75 ^a	5.00 ^a	59.97 ^a	1033.77 ^b
2	148	2.96 ^{ab}	0.88 ^a	4.33 ^c	56.54 ^{ab}	1016.22 ^b
3	90	2.93 ^{bc}	0.58 ^b	4.45 ^b	50.19 ^b	1124.44 ^a
4	163	2.90 ^c	0.83 ^a	3.97 ^d	53.94 ^{ab}	981.60 ^b
P-value		0.000	0.005	0.000	0.736	0.004
Season						
Spring	70	3.00	0.80 ^a	5.00	74.30 ^a	800 ^b
Summer	253	2.94	0.72 ^b	5.00	54.25 ^b	1000 ^a
Autumn	40	3.00	0.62 ^c	4.58	50.10 ^b	800 ^b
Winter	115	3.00	0.30 ^d	5.00	52.17 ^b	1160 ^a
P-value		NS	<0.05	NS	<0.05	<0.05

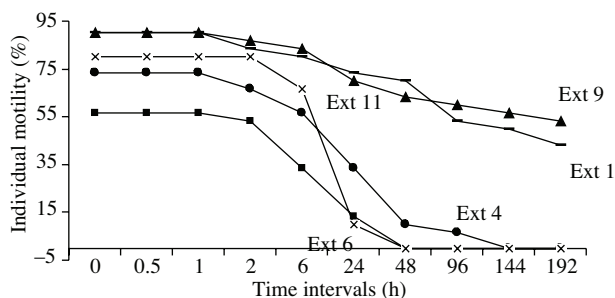


Figure. Changes in the individual motility of the semen with various extenders at different postdilution intervals. Legend: extender 1–; 4 •; 6 ■; 9 ▲; and 11 x. The extenders were different in retaining semen motility during various time intervals; F = 13.437, P = 0.000.

sperm motility for more than 192 h, while extender 6 (27.00%) had the lowest postdilution spermatozoa motility (Figure). The extender containing 20% egg yolk and 7% glycerol maintained sperm motility for more than 192 hrs (72.33%), followed by 15% egg yolk and 5% glycerol (58.33%) and by 25% egg yolk and 10% glycerol (46.33%), and the lowest sperm motility was observed with the extender containing no glycerol but 20% egg yolk (39.67%).

3.5. Relationship among various parameters

Table 3 reports the relationship among various quality parameters. The time of collection correlated significantly with the semen volume and the semen collected during the early morning resulted in the best semen volume, which significantly decreased with the delay (r = -0.237,

P = 0.000). The semen volume also correlated positively with the libido and negatively with the mass motility and concentration. The concentration correlated positively with the time of collection and the mass motility. The individual and mass motility correlated positively with each other.

4. Discussion

4.1. Effect of month on the semen quality

The month significantly affected the libido, semen volume, mass motility, and individual motility. The decline in libido during June, mass motility from November to January, and individual motility from November to January and May to June may be the manifestations of seasonal stress. The higher individual motility during March and July to October may be due to the favorable effect of spring and autumn and the availability of green pastures in abundance. Volume showed the highest value during January (1.60 mL). Sperm concentrations were highest during November, followed by June. This may be due to the environmental effects on animal physiology associated with sexual behavior and spermatogenesis, which are affected by the months.

In a previous study, 23 purebred Alpine (n = 8), Saanen (n = 7), and Damascus (n = 8) goat bucks raised in Thessaloniki, Greece, were used to monitor the effect of photoperiod on semen production (8). The best semen was produced during the breeding season (late summer and autumn). However, the magnitude of these seasonal effects was not sufficient to prevent bucks from being used

Table 3. Relationship among the various parameters of the semen quality in dairy bucks (r = Pearson’s correlation coefficient, P = probability values, and n = 480).

Parameters	Statistical value	TColl ¹	Lib	Vol	MM	Ind M
Lib	R	0.057	-	-	-	-
	P	0.323	-	-	-	-
Vol	R	-0.237	0.136	-	-	-
	P	0.000	0.003	-	-	-
MM	R	0.003	-0.128	-0.336	-	-
	P	0.952	0.005	0.000	-	-
Ind M	R	0.043	0.072	0.023	0.176	-
	P	0.462	0.116	0.614	0.000	-
Conc	R	0.144	0.053	-0.342	0.129	-0.055
	P	0.012	0.242	0.000	0.005	0.234
	N	303	480	480	480	478

¹TColl: time of collection (0800–1100 hours); Lib: libido; Vol: volume; MM: mass motility; Ind M: individual motility; Conc: concentration.

for breeding throughout the year. Nevertheless, individual differences in the semen quantity and quality among bucks within a breed make the individual evaluation of semen necessary to select the most fertile males for breeding.

Ten Zaraibi buck goats were used to study the effect of season on semen quality (9). Bucks demonstrated the highest libido in the summer, using the least ($P < 0.01$) number of mounts and a shorter ($P < 0.01$) reaction time. This coincided with a higher ($P < 0.01$) level of plasma testosterone (10 ng/mL) and the best semen quality (semen index: 3354×10^6). During autumn, the highest ($P < 0.01$) ejaculate volume (0.98 mL) and the maximum output of sperm (4565×10^6), as well as a lower ($P < 0.01$) percentage of sperm abnormalities (8.8%), were recorded. This was accompanied by high concentrations of plasma luteinizing hormone (average: 2.9 mIU/mL).

Öztürkler (10) used 6 Kıvrıkcık rams in Turkey to investigate the semen quality in different seasons. The season affected the semen volume, increasing during the breeding season, but the other quality parameters were not affected. Postthaw motility of the semen during the breeding season was higher and the acrosomal and total morphological abnormalities were lower than in the nonbreeding season ($P < 0.05$). In a previous study, seasonal variations of sexual activity were studied in local bucks (11). In conclusion, bucks of a local breed in western Algeria have seasonal variations of sexual activity in relation to the annual photoperiod variation; short days stimulate the sexual activity, whereas long days inhibit it.

4.2. Semen productivity potential

The parameters recorded in this study comprising libido, semen volume, mass motility, individual motility, and sperm concentration were satisfactory as indicators of the semen productivity potential of the local dairy goats. The semen may be considered for processing and AI under field conditions.

Seminal plasma contributes to about 70% of the volume of the ejaculate. Therefore, when the volume increases or decreases, the changes are mainly due to changes in the quantities of fluids secreted by the epididymis and the accessory glands (12). In the present study, the semen quality was satisfactory, which may be the result of better functioning of the accessory glands supported by a good body condition score, feeding, grazing, and enough exercise.

4.3. Effect of extenders

In the present study, the extender combination of high fructose with high glycerol or lower fructose with lower glycerol protected the spermatozoa motility for up to 192 h after dilution compared to other extenders. Sugar maintains the osmotic pressure of the diluents by inducing cell dehydration and less ice crystal formation into the

spermatozoa (13). Higher levels of glycerol have been reported to increase the number of spermatozoa with damaged acrosomes (14), which may have been prevented in the present study by the higher levels of fructose in the second combination. Yildiz et al. (15) have already reported the protective effect of fructose on spermatozoa.

Graham (16) observed a negative effect of glycerol on the semen quality of bucks. Neither the removal of seminal plasma nor the type of extender had any effect on the semen quality before freezing, but semen frozen in a Tris-citric acid-glucose buffer with egg yolk without removal of the seminal plasma had better quality after thawing than the semen frozen in other diluents or after removal of seminal plasma (17).

According to Aboagla and Terada (18), it was valuable to include sugars in cryopreservation diluents, as seminal plasma contains sugars. Goat sperm readily utilizes fructose, glucose, lactose, and other sugars for respiration and these sugars also provide osmotic balance and cryoprotection, but of all the sugars, fructose has the greatest molar concentration in goat semen.

The present study showed that any alteration in the percentage of egg yolk and glycerol beyond certain limits resulted in a drastic decline in sperm motility, while the absence of fructose resulted in the loss of potency of the sperm. According to Ritar and Salamon (19) and Tuli and Holtz (20), the most common nonpenetrating cryoprotectant used was egg yolk (2%–20%). Ritar and Salamon (19) reported that the pH of the egg yolk-based or milk-based goat sperm diluents should normally range between 6.75 and 6.8.

4.4. Semen survivability

Storage of the semen for longer periods causes ultrastructural, biochemical, and functional damage to the spermatozoa, resulting in a reduction of motility, viability, and fertility and impaired transport (21). The changes were attributed to certain sperm abnormalities or exposure to external environmental conditions, and adjustment to the sudden changes in the environment resulted in the reduction of sperm motility.

The sperm motility in the present study declined throughout the liquid storage and the drastic reduction occurred during the first 24 h after collection. Our findings are supported by Vaughan et al. (22), who reported 10% and 5% reductions in sperm motility between 24 and 48 h of liquid storage in Biladyl® and Triladyl® diluents, respectively.

5. Conclusion

In the present study, the extender combination of high fructose with high glycerol or lower fructose with lower glycerol protected the spermatozoa motility for up to 192

h after dilution compared to other extenders. The libido showed a decline during June and the mass motility declined during November to January. The individual motility remained higher during March and July to October. The volume showed the highest value during January (1.60 mL). Sperm concentrations were the highest during November, followed by June.

References

1. Kareta, W., Smor, Z., Gajda, B., Jura, J., Ceg, M.: Estrus synchronization and superovulation in goats. *Ann Anim Sci—Rocz. Nauk Zoot*, 1999; 26: 163–171.
2. Qureshi, M.S.: Final Report, Genetic Improvement of Dairy Goats in NWFP, conducted under Pakistan Science Foundation Grant No. R&D/CDYST/F-VRI/Bio (20). KP Agricultural University, Peshawar, 2006.
3. Leboeuf, B., Restall, B., Salamon, S.: Production and storage of goats semen for artificial insemination. *Anim. Reprod. Sci.*, 62: 113–141.
4. Abdelhakeam, A.A, Graham, E.F., Vazquez, I.A.: Studies on the presence and absence of glycerol in unfrozen and frozen ram semen: fertility trials and the effect of dilution methods on freezing ram semen in the absence of glycerol. *Cryobiology*, 1991; 28: 36–42.
5. Hervieu, J., Morand, F.P., Schmidely, P., Fedele, V., Delfa, R.: Body measurements explaining variations in scores for the sternal, lumbar, and caudal regions used to estimate body condition in dairy goats. *Options Méditerranéennes—Série Séminaires*, 1991; 13: 43–56.
6. SPSS, Inc.: SPSS, Version 10.0. SPSS, Chicago, IL, USA. 1999.
7. Steel, R.G.D., Torrie, J.H.: *Principle and Procedure of Statistics*. McGraw Hill, New York. 1981.
8. Karagiannidis, A., Varsakeli, S., Karatzas, G.: Characteristics and seasonal variations in the semen of Alpine, Saanen and Damascus goat bucks born and raised in Greece. *Theriogenology*, 2000; 53: 1285–1293
9. Barkawi, A.H., Elsayed, E.H., Ashour, D., Shehata, E.: Seasonal changes in semen characteristics, hormonal profiles and testicular activity in Zaraibi goats. *Small Rumin. Res.*, 2006; 66: 209–213.
10. Öztürkler, Y., Ak, K., İleri, İ.K.: The effect of season on spermatological characteristics of frozen-thawed semen in Kivırcık rams. *Kafkas Univ Vet Fak Derg.*, 1997; 1: 73–79.
11. Hammoudi, S.M., Ait-Amrane, A., Belhamiti, T.B., Khiati, B., Niar, A., Guetarni, D.: Seasonal variations of sexual activity was studied in local bucks in western Algeria. *Afr. J. Biotechnol.*, 2010; 9: 362–112.
12. Mann, T.: *The Biochemistry of Semen*. Methuen and Co. Ltd., London. 1954.
13. Purdy, P.H.: A review on goat sperm cryopreservation. *Small Rumin. Res.*, 2006; 63, 215–225.
14. Deka, B.C., Rao, A.R.: Effect of glycerol level in Tris-base extender and equilibration period on quality of frozen goat semen. *Theriogenology*, 1986; 26: 231–238.
15. Yildiz, C., Kaya, A., Aksoy, M., Tekeli, T.: Influence of sugar supplementation of the extender on motility, viability and acrosomal integrity of dog spermatozoa during freezing. *Theriogenology*, 2000; 54, 579–585.
16. Graham, J.K.: Assessment of sperm quality: a flow cytometric approach. *Anim. Reprod. Sci.*, 2001; 68: 239–247.
17. Peterson, L., Nanda, K., Opoku, B.K., Ampofo, W.K., Owusu-Amoako, M., Boakye, A.Y., Rountree, W., Troxler, A., Dominik, R., Roddy, R., Dorflinger, L.: SAVVY® (C31G) Gel for prevention of HIV infection in women: a phase 3, double-blind, randomized, placebo-controlled trial in Ghana. *PLoS ONE*, 2007; 2: 1–8.
18. Aboagla, E.M.E., Terada, T.: Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. *Biol. Reprod.*, 2003; 69: 1245–1250.
19. Ritar, A.J., Salamon, S.: Effects of seminal plasma and its removal and of egg yolk in the diluent on the survival of fresh and frozen thawed spermatozoa of the Angora goat. *Aust. J. Biol. Sci.*, 1982; 35: 305–312.
20. Tuli, R.K., Holtz, W.: Effect of glycerolization procedure and removal of seminal plasma on post-thaw survival and GOT-release from Boer goat spermatozoa. *Theriogenology*, 1994; 42: 547–555.
21. Waberski, D., Schapmann, E., Henning, H., Riesenbeck, A., Brandt, H.: Sperm chromatin structural integrity in normospermic boars is not related to semen storage and fertility after routine AI. *Theriogenology*, 2011; 75: 337–345.
22. Vaughan, J.L., Galloway, D., Hopkins, D.: *Artificial insemination in alpacas (Lama pacos)*. Rural Industries Research and Development Corporation, Kingston, ACT, Australia. 2003.

Acknowledgments

The authors are thankful to the Pakistan Science Foundation for sponsoring this study under Grant No. PSF (Bio) 403, and the Charles Sturt University in Australia is acknowledged for providing visiting/adjunct professorship to the first author and access to the IT and library resources. Dr Yousaf Hayat assisted us in the statistical analysis.