

# Relationship of Extender and Packaging System an the Length of Preservation and the Quality of Chilled Semen of Boer Goat

Febretrisiana A, Anwar, Sinulingga S

Indonesian Goat Research Station, PO Box I Sei Putih, Galang 20585, North Sumatra

(received 22-01-2016; revised 08-03-2016; accepted 16-03-2016)

## ABSTRAK

Febretrisiana A, Anwar, Sinulingga S. 2016. Hubungan jenis pengencer dan sistem pengemasan terhadap lama simpan dan kualitas semen cair kambing Boer. JITV 21(1): 49-54. DOI: <http://dx.doi.org/10.14334/jitv.v21i1.1350>

Tujuan penelitian ini adalah untuk membandingkan penggunaan bahan pengencer yang berbeda (Triladyl dan Tris kuning telur (TKT)) serta pengaruh cara pengemasan semen cair (pool dan straw) terhadap kualitas semen cair kambing Boer. Semen ditampung menggunakan vagina buatan yang berasal tiga ekor kambing pejantan Boer berumur 2 tahun dengan bobot hidup 50-55 kg. Semen dievaluasi kemudian diencerkan dengan menggunakan pengencer Triladyl dan TKT kemudian disimpan di lemari pendingin dengan suhu 5°C dalam kemasan yang berbeda yaitu dalam bentuk pool dan straw. Evaluasi dilakukan setiap hari selama 5 hari. Hasil penelitian menunjukkan motilitas sperma yang lebih baik diperoleh dengan menggunakan pengencer Triladyl dan dengan sistem pengemasan pool, lebih tinggi ( $P<0,05$ ) bila dibandingkan menggunakan sistem pengemasana straw dan lebih tinggi bila menggunakan pengencer TKT dengan kedua sistem penyimpanan (masing-masing 45,8%, 26,1%, 32,1% dan 9,1%). Hal sama juga terlihat pada integritas membran spermatozoa yang terlihat lebih tinggi ( $P<0,05$ ) bila menggunakan pengencer Triladyl baik dengan sistem penyimpanan pool (75,2%) maupun dengan straw (77,2%). Viabilitas spermatozoa dengan menggunakan pengencer Triladyl baik dengan pengemasan pool maupun straw mulai mengalami penurunan setelah 3 hari penyimpanan (77,1% dan 76,2%;  $P<0,05$ ) sedangkan dengan pengencer TKT viabilitas spermatozoa menurun setelah 4 hari penyimpanan (73,2% dan 58,0%;  $P<0,05$ ). Kesimpulan dari penelitian ini adalah kualitas semen cair kambing Boer menurun seiring dengan bertambahnya waktu penyimpanan dan pengencer Triladyl dengan sistem pengemasan pool menjadi metode terbaik untuk preservasi semen cair kambing Boer.

**Kata Kunci:** Semen Cair, Boer, Triladyl, Tris Kuning Telur, Straw

## ABSTRACT

Febretrisiana A, Anwar, Sinulingga S. 2016. Relationship of extender and packaging system an the length of preservation and the quality of chilled semen of Boer goat. JITV 21(1): 49-54. DOI: <http://dx.doi.org/10.14334/jitv.v21i1.1350>

The aim of this research was to compare the effectiveness of different extender (either Triladyl or Tris Egg Yolk extender) and different packaging method (pool and straw) of chilled semen an the length of preservation and the quality of chilled semen of Boer goat. Semen was collected using an artificial vagina from 3 two years old Boer bucks with body weight of 50-55 kg. It was evaluated under a microscope, then each was diluted either in Tris egg yolk extender (TEY) or Triladyl. Those diluted sperms were then packed either in pool or straw and preserved at 5°C refrigerator. Sperm motility, viability and membrane integrity of each group were evaluated every 24 h for up to 5 days. Results showed that sperm motility in Triladyl of pool packaging system up to 3 days was higher than straw packaging system or TEY in pool or straw packaging system which were 45.8%, 26.1%, 32.1% and 9.1%, respectively ( $P<0.05$ ). Percentage of sperm membrane integrity showed the same pattern to Triladyl both in pool and straw packaging system which was higher than TEY group (75.2% and 77.2%;  $P<0.05$ ). Sperm viability in Triladyl both in pool or straw packaging system decreased ( $P<0.05$ ) after 3 days of preservation (77.1% and 76.2%) but TEY significantly decreased after 4 days of preservation either in pool or straw packaging system (73.2% and 58.0%;  $P<0.05$ ). It was concluded that sperm quality decreased with increasing of the length of preservation while Triladyl extender in pool packaging system showed the best quality.

**Key Words:** Chilled Semen, Boer, Triladyl, Tris Egg Yolk, Straw

## INTRODUCTION

Artificial insemination program is one of reproduction technologies aimed to increase pregnancy rate in animal. It encourages crossing program of selected buck. The artificial insemination is used intensively in goat breeding system, especially in

crossbreeding program to increase meat or milk yield and litter size. It was also used to optimize selection program and facilitate to control time of birth (Leboeuf et al. 2000). Generally, artificial insemination use frozen semen. Limited liquid nitrogen due to distribution or evaporation is an issue in frozen semen use. Limited container availability and late in reporting estrus also affect the artificial insemination performance

using frozen semen. Chilled semen use in artificial insemination became an alternative to anticipate those issues (Leethongdee 2010).

Preservation process of chilled semen was commonly performed at 4-5°C with limited shelf life by 24-48 h after collection (Mara 2007). Extender material is one of important factors determining success of chilled semen preservation. Extender material is materials added to assure physical and chemical requirement for spermatozoa, raise the volume, and protect the spermatozoa from a cold shock. Extender material could be used as nutrient resource of spermatozoa, preventing growth of germs and maintaining osmotic tension and electrolyte balance to keep spermatozoa alive (Ismaya 2014). Extender of semen should contain similar contents with physical and chemical properties of plasma semen, did not contain toxic substance, and did not restrict fertility ability of spermatozoa (Verberckmoes et al. 2004).

Environment also affects chilled semen quality of goat preserved at 5°C in long term. In oxygenated environment, spermatozoa have shorter vitality. This is related to function of the oxygen as oxidative element in metabolic producing harmful waste product of metabolic oxidation such as hydrogen peroxide. Salomon & Maxwell (2000) reported that lipid peroxide played a key role in aging process; decreasing spermatozoa vitality; inducing structure change especially in acrosome area; damage in spermatozoa structure; biochemical and functional damage including decrease of spermatozoa motility, membrane integrity, or fertilization ability.

This study was aimed to evaluate effect of preservation of chilled semen of Boer goat using different extender and preservation method against quality and vitality of spermatozoa preserved at 5°C. This study results were expected to solve technical issues in the field, simplifying and accelerating success of artificial insemination in goat in Indonesia.

## MATERIALS AND METHODS

This study was conducted in Laboratory of Reproduction of Indonesian Goat Research Station, Sei Putih, North Sumatera. This study was conducted for 6 months (February-July 2015).

### Sample of animal

In this study three two year old Boer bucks with body weight of 50-55 kg were used. Cage system and diet provision were conducted individually and with ad libitum of water.

### Semen collection, process, and sperm preservation

Fresh semen was evaluated in laboratory. Semen dilution used two different extenders, Triladyl (commercial) and Tris Egg Yolk (TEY) with composition of aminomethane by 2.96 mL, yolk by 2 mL, citrate acid by 1.65 g, lactose by 2.16 mL, glycerol by 6 mL, penicillin and streptomycin by 1000 IU/mL, aquabidest ad by 100 mL. Concentration of final spermatozoa was  $200 \times 10^6/\text{mL}$ . Semen was preserved in 5°C refrigerator in 15 mL tube (pool) and 0.25mL straw. Examination of vitality of chilled semen by calculating percentage of motility, viability, and membrane integrity in day-0, 1, 2, 3, 4, and 5 of preservation.

### Evaluation of motility

Percentage of spermatozoa motility was determined by observation of progresively moved soermatozoa conducted subjectively in 6 different points of view. Score provided was from 0% (there was no spermatozoa moved) to 100% (all spermatozoa moved forward).

### Evaluation of membrane integrity

Membrane integrity of spermatozoa was evaluated using Hypoosmotic Swelling Test (HOS-Test) in 5 points of view randomly with circular tail spermatozoa (whole membrane plasma) or straigh tail spermatozoa (incomplete membrane plasma). Total number of spermatozoa calculated was 200 spermatozoa.

### Evaluation of viability

Percentage of viability was percentage of life and death spermatozoa using eosin-negrosin staining method. Spermatozoa was cathegorized as life if the head was colorless and cathegorized as death if the head was red. Percentage of viability of spermatozoa was determined according to comparison of the number of life spermatozoa and total amount of spermatozoa. Total amount of spermatozoa counted was 200 heads.

### Data analysis

Data of viability and quality of chilled semen preserved at 5°C in the two groups were in percentage and analyzed using analysis of variance (ANOVA). This study was in completely randomized design with 5 replications. The analysist was continued by Duncan's Multiple Range Test (DMRT) if there was a difference between the groups.

## RESULT AND DISCUSSION

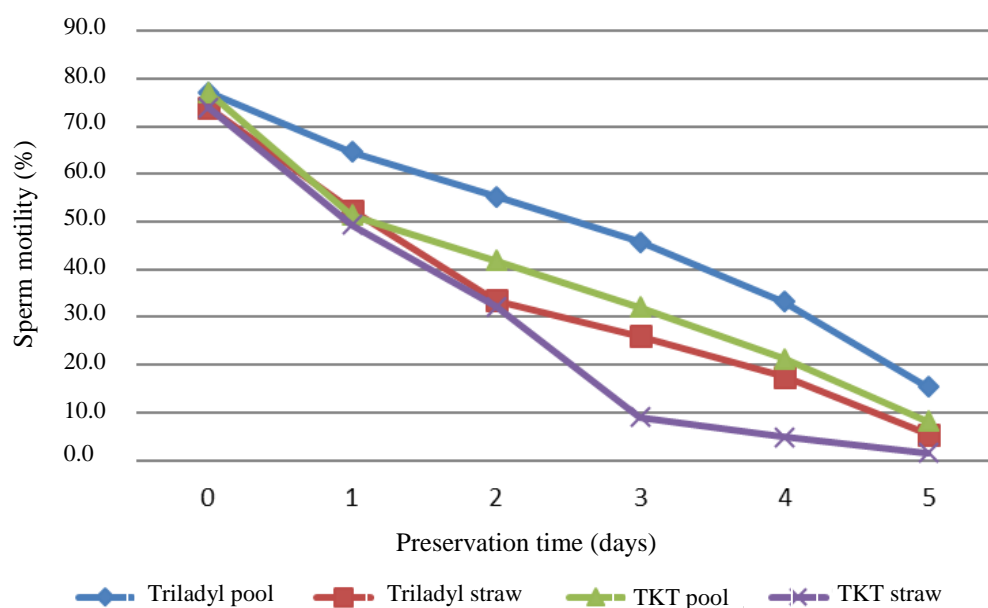
### Sperm motility

Result showed that use of Trilady extender with pool packaging showed higher motility rate ( $P<0.05$ ) up to day-5 of preservation than other groups (77.1%, 64.6%, 55.3%, 45.8%, 33.2% and 15.3% respectively at day 1, 2, 3, 4 and 5 of preservation) showed in Figure 1. Straw packaging of Trilady extender significantly ( $P<0.05$ ) decreased motility rate since the first day of preservation (52.4%) to day-2 preservation (33.6%). Use of TEY extender showed lower motility both with pool and straw packaging system. Each only could maintain the quality of spermatozoa up to day-2 of preservation (41.9%). Quality of spermatozoa in day-1 of preservation was 49.3% ( $P<0.05$ ). Use of Triladyl with pool packaging in day-3 of preservation showed feasible percentage for the artificial insemination.

It was showed that Triladyl extender with pool packaging system was able to maintain spermatozoa motility up to day-3 of preservation. Motility rate in all groups decreased along with increase of preservation time. Decrease of motility was assumed due to damage of spermatozoa during the preservation at low temperature ( $5^{\circ}\text{C}$ ). Chilling was one way to slow down cell metabolism to maintain cell viability. It was also able to decrease oxygen requirement and slow down acid accumulation as a result of apoptosis. Molecular activities and ion mobility regulated by thermal energy were basic of biological and chemical process. Decrease in thermometer slowed down the molecule activities.

Biochemical process could not be separated from interaction process between molecules in catalyst reactions by enzyme and chilling method strongly influenced all those reaction components (Taylor 2006).

Addition of extender might protect spermatozoa from cold stock. Tris Egg Yolk was able to protect spermatozoa from cold sock, so that was used as basic material of extender (Alves et al. 2013). Low density lipoprotein (LDL) in Tris Egg Yolk consisted of 79% lipid and 21% protein with the main component was cholesterol. Structure of lipoprotein in Tris Egg Yolk was similar with structure of plasma membrane and it could protect the spermatozoa (Botham & Mayes 2009). However, in this study, Triladyl use showed better result than TEY. Fructose component in Triladyl extender played a role to maintain spermatozoa motility during preservation compared to lactose component in TEY extender. Other indication was caused by different energy use path in cell of each sugar was different related to metabolism of nitrogen (Medrano 2006). Fructose was included in to easily changed sugar in to energy source (Schorin 2012). Addition of fructose might be energy source for spermatozoa as known it was also be the main energy source in seminal plasma (Stefanov 2015). Fructose might also protect sperm from cold shock both inside and outside the cell. This was caused by sugar with big molecule size might serve as intracellular or extracellular cryoprotectant, so that fructose might enter intracellular cryoprotectant and the fructose might penetrate into cell (Klinc & Rath 2006; Paulenz et al. 2009).

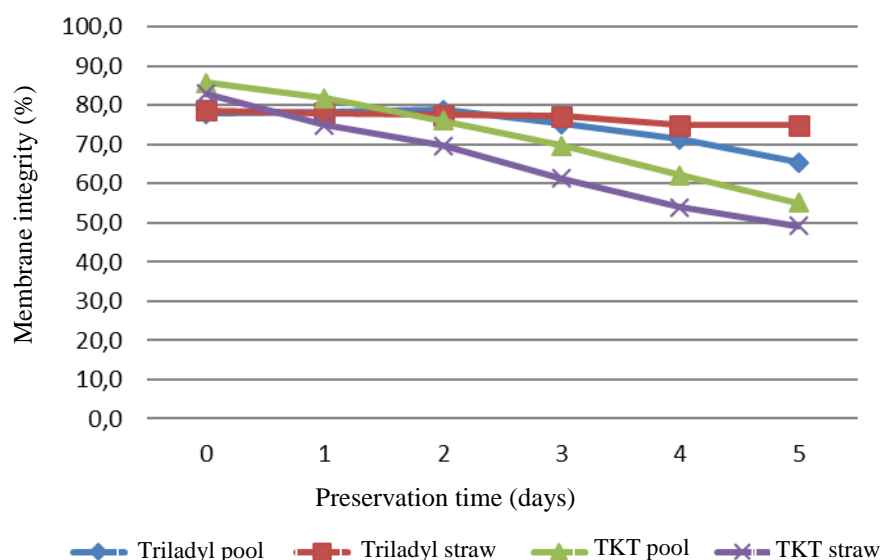


**Figure 1.** Motility rate of boer goat spermatozoa by different extender and packaging system and preserved at  $5^{\circ}\text{C}$  for 5 days.

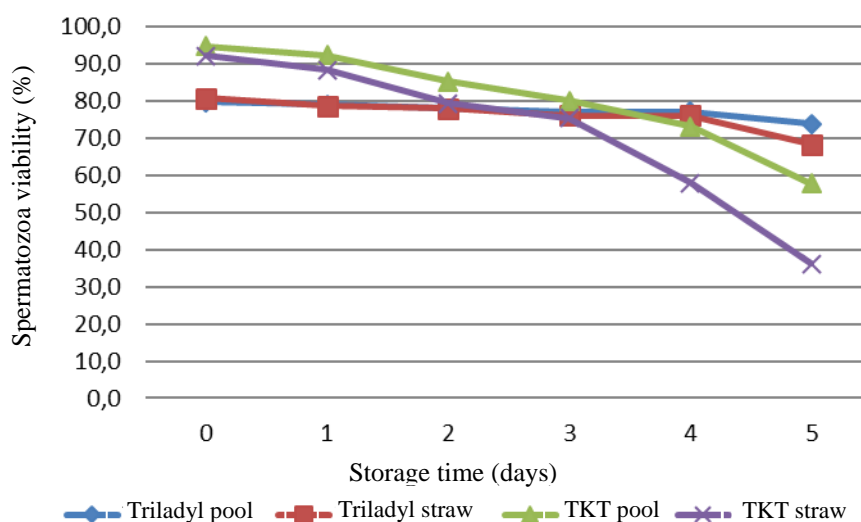
### Integrity of spermatozoa cell membrane

Generally, integrity of spermatozoa cell membrane of Boer goat preserved at 5°C showed high presentation using Triladyl with both pool or straw packaging method. Difference of integrity rate of membrane in the 4 groups started on the day-2 of preservation. Integrity of spermatozoa membrane using TEY extender with both pool and straw packaging method significantly ( $P<0.05$ ) decreased up to day-5 of preservation. Integrity membrane rate using Triladyl extender was

still good up to day-5 of preservation both with pool (65.5%) or straw (75.0%) packaging (Figure 2). Different result ( $P<0.05$ ) was showed by the group using TEY both with pool (55.1%) or straw (49.2%) packaging method which showed lower integrity rate of spermatozoa membrane and was not feasible for artificial insemination. Recommended integrity of spermatozoa membrane feasible for the artificial insemination was  $\geq 60\%$  (Revel & Mrode 1994).



**Figure 2.** Integrity rate of spermatozoa membrane of Boer goat with different extender and packaging system preserved at 5°C for 5 days.



**Figure 3.** Viability rate of Boer goat with different extender and packaging system preserved at 5°C for 5 days.

Figure 2 shows that percentage of integrity of spermatozoa membrane added by Triladyl both with pool and straw packaging method is higher than other group. It was known that spermatozoa viability was related to integrity of spermatozoa membrane. It was possible that Triladyl extender able to maintain plasma membrane requirement during preservation. It was due to plasma membrane played a role in managing in and out traffic of all substrate and electrolyte needed in metabolic process (Ariantje 2014). Damage in plasma membrane might affect motility and causing death in semen due to aspartate aminotransferase enzyme (AspAT) which was the primary enzyme in mitochondria producing ATP would be released from cell into seminal plasma which would interfere ATP production (Arifiantini & Purwantara 2010).

### Sperm viability

Percentage of sperm viability using Triladyl extender started to significantly ( $P < 0.05$ ) decrease in day-3 of preservation both with pool (77.1%) and straw (76.2%) packaging method (Figure 3). Therefore, viability of spermatozoa using TEY extender both with pool or straw packaging decreased after day-4 of preservation. Figure 3 shows that decrease of sperm viability percentage using TKT both with pool and straw packaging is significantly ( $P < 0.05$ ) lower than sperm viability percentage using Triladyl in day-3 of preservation (73.2% and 58.0%, respectively). The lowest ( $P < 0.05$ ) viability was showed by spermatozoa preserved by TEY extender with straw method after day-5 of preservation (36.1%).

Figure 3 shows that Triladyl was able to maintain spermatozoa better than TEY extender. Yolk concentration was lower in Triladyl extender than the Tris. It was assumed that Tris played a role in maintaining spermatozoa viability. Holt et al. (1996) reported that higher concentration of yolk would give negative effect to motility and viability. Especially in goat, phospholipase A enzyme in plasma semen secreted by bulbo-urethralis gland would easily damage semen extender medium especially the one containing of yolk. It caused death of spermatozoa. High concentration of lysolecithin from hydrolysis of yolk lecithin through phospholipase A enzyme snapping in spermatozoa environment caused toxic effect in goat spermatozoa. Leboeuf et al. (2000) said that bulbo-urethralis gland (Cowper gland) of buck synthesized an enzyme and secreted in plasma semen where coagulation might be occurred if it interacted with yolk. This enzyme was identified as phospholipase A which might hydrolyze lecithin of yolk into fatty acid and lysolecithin poisoning for spermatozoa. Change of spermatozoa viability during preservation for a long term was also strongly related to condition of

mitochondria membrane of spermatozoa cell (Love et al. 2003). Mitochondria membrane would damage if there was a composition change in phospholipid and if there was a temperature fluctuation during incubation (Kasimanickam et al. 2012).

The result of this study showed that generally, Triladyl extender was able to maintain chilled semen longer than TEY extender. This was assumed due to the commercial Triladyl extender had better and more complete composition than TEY extender. The result also showed that pool packaging method was better than the straw. It might be caused by difference of ratio of amount extender and concentration or amount spermatozoa in the straw and pool. Concentration of spermatozoa in extender influenced the competition of spermatozoa in nutrient consumption. Metabolism persisted during preservation and causing accumulation of lactic acid increasing pH of medium and causing damage and degrading quality of spermatozoa along with longer time for preservation (Munsi et al. 2007).

### CONCLUSION

Triladyl extender with pool packaging was the best method to maintain chilled semen quality of Boer goat which could persist up to day-3 of preservation at 5°C.

### ACKNOWLEDGEMENT

Author thanked to Fitra Aji Pamungkas, Rian Rosartio, and technicians who helped during this study.

### REFERENCES

- Ariantje OS, Yusuf TL, Sajuthi D, Arifiantiny RI. 2014. Kualitas semen cair kambing Peranakan Etawah dalam modifikasi pengencer tris dengan trehalosa dan rafinosa. *J Vet.* 15:11-22.
- Arifiantini RI, Purwantara B. 2010. Motility and viability of Fresian Holstein spermatozoa in three different extender preserved at 5°C. *J Indones Trop Anim Agric.* 35:222-226.
- Alves HM, Oliveira IR, Castelo TS, Lima GL, Souza AL, Moreira AL, de Paula VV, Silva AR. 2013. Comparison of different glycerol and egg yolk concentrations added to tris-based extender for the collared peccaries (*Tayassu tajacu*) semen freezing. *Reprod Domest Anim.* 48:506-511.
- Botham KM, Mayes PA. 2009. Pengangkutan dan penyimpanan lipid. In: Murray RK, Granner DK, Rodwell VW, editors. *Harper's Illustrated Biochemistry*. 27th ed. Pendit BU, penerjemah. Jakarta (Indones): EGC. p. 225-238.
- Holt WV, Abaigar T, Jabbour HN. 1996. Oestrus synchronization, semen preservation and artificial

- insemination in the Mohor Gazelle (*Gazella dama mhorr*) for the establishment of a genome resource bank programme. *Reprod Fertil Dev.* 8:1215-1222.
- Ismaya. 2014. Bioteknologi inseminasi buatan pada sapi dan kerbau. Yogyakarta (Indones): Gadjah Mada University Press.
- Klinc P, Rath. 2006. Application of flowcytometrically sexed spermatozoa in different farm animal species: a review. *Archiv Tierzuch Dummerstorf.* 49:41-54
- Kasimanicham VR, Kasimanicham RK, Memon MA. 2012. Effect of extenders on sperm mitochondrial membrane, plasma membrane and sperm kinetics during liquid preservation of canine semen at 5°C. *J Amin Reprod Sci.* 136:139-145.
- Leboeuf B, Restall B, Salamon S. 2000. Production and storage of goat semen for artificial insemination. *Anim Reprod Sci.* 62:113-141.
- Leethongdee S. 2010. Development of trans-cervical artificial Insemination in sheep with special reference to anatomy of cervix. *Suranaree J Sci Technol.* 17:157-169.
- Love CC, Thompson JA, Brinsko SP, Rigby SL, Blanchard TL, Lowry VK, Varner DD. 2003. Relationship between stallion sperm motility and viability as detected by two fluorescence staining techniques using flow cytometry. *Theriogenology.* 60:1127-1138.
- Mara L, Dattena M, Pilichi S, Sanna D, Branca A, Cappai P. 2007. Effect of different extender on sperm motility: collection and goat semen fertility, evaluation of semen. *Anim Reprod Sci.* 102:152-157.
- Medrano A, Garcia-Gill N, Ramio L, Montserrat RM, Pinart E, Concha II, Bonet S, Rigau T, Rodriguez-Gill JE. 2006. Hexose-specificity of hexokinase and ADP-dependence of pyruvate kinase play important roles in the control of monosaccharide utilization in freshly diluted boar spermatozoa. *Mol Reprod Dev.* 73:1179-1194.
- Munsi MN, Buiyan MMU, Alam MGS. 2007. Effects of exogenous glutathione on the quality of chilled bull semen. *Reprod Domest Anim.* 42:358-362
- Paulenz H, Soderquist L, Perez R, Berg KA. 2009. Effect of different extenders and storage temperature on sperm viability of liquid ram semen. *Theriogenology.* 57:823-836.
- Revell SG, Mrode RA. 1994. An osmotic resistance test for bovine semen. *Anim Reprod Sci.* 36:77-86.
- Salomon S, Maxwell WMC. 2000. Preservation of ram semen. *Anim Reprod Sci.* 62:77-111.
- Stefanov RG, Anev G, Abadjieva DV. 2015. Effect of different extenders and storage periods on motility and fertility of ram sperm. *Mac Vet Rev.* 38:85-89.
- Schorin, Marilyn D, Sollid, Kris RD, Edge, Marianne MS, Bouchoux, Ann MSW. 2012. The science of sugar part 1: A closer look at sugars. *Nurt Today.* 3:96-101.
- Taylor MJ. 2006. Biology of cell survival in the cold: The basis for biopreservation of tissues and organs. *CRC-Taylor & Francis.* p. 15-62.
- Verberckmoes S, Van Soom A, Dewulf J, De Pauw I, de Kruif A. 2004. Preservation of fresh bovine semen in extender based on the ionic composition of cauda epididymal plasma. *Reprod Domest Anim.* 39:1-7.