Arq. Bras. Med. Vet. Zootec., **v.65, n.1, p.6-12, 2013**

Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen

[*Caracrterísticas espermáticas e bioquímicas do plasma seminal de caprinos nas estações chuvosa e seca do Nordeste brasileiro: influência da estação no resfriamento do sêmen*]

G.V. *Aguiar*[1], M.F. *van Tilburg*[1], A.G.V. *Catunda*[1], C.K.S. *Celes*[1], I.C.S. *Lima*[1], A.C.N. *Campos*[1], A.A.A. *Moura*[1*], A.A. Araújo[2]

[1]Universidade Federal do Ceará – Fortaleza, CE
[2]Universidade Estadual do Ceará – Fortaleza, CE

ABSTRACT

The present study aimed to verify the caprine semen characteristics during dry and rainy seasons in the Brazilian Northeast, and the influence of these seasons on cooled semen. Seminal volume, concentration, percentage of motile cells, vigor and spermatic morphology, as well as biochemical profile (fructose, citric acid, P, Ca^{2+}, Mg, total proteins and phospholipase A_2 activity) were analyzed. It was observed a reduction ($P<0.05$) in normal sperm morphology, fructose, citric acid, P, Mg and total protein concentration during the dry season, which did not affect the motility, vigor, volume and sperm concentration. Phospholipase A_2 activity was increased during the dry season ($P<0.05$). The analysis of the semen cooled at 4ºC during 48 hours showed reduction in total motility and vigor sperm during the dry season ($P<0.05$). Based on these results, we conclude that the best period of year for caprine semen cooling is the rainy season.

Keywords: semen, spermatozoa, season, cooling, caprine

RESUMO

Verificou-se as características seminais de caprinos durante a época seca e a chuvosa no Nordeste brasileiro e a influência da época no resfriamento do sêmen. Foram mensurados volume, concentração espermática, porcentagem de espermatozoides móveis, vigor, morfologia espermática e características bioquímicas (frutose, ácido cítrico, fósforo, magnésio, proteínas totais e atividade da fosfolipase A_2). Observou-se redução ($P<0,05$) no número de espermatozóides morfologicamente normais, frutose, ácido cítrico, fósforo, magnésio e proteínas totais durante a época seca que não influenciaram na motilidade, vigor, volume e concentração do sêmen. Entretanto, a atividade da fosfolipase A_2 foi maior na época seca. Quando o sêmen foi submetido ao resfriamento a 4°C durante 48 horas, houve redução ($P<0,05$) na motilidade total e no vigor espermático durante a época seca. Com base nesses resultados, conclui-se que o período chuvoso é melhor para resfriar sêmen de caprinos no Nordeste brasileiro.

Palavras-chave: sêmen, espermatozoide, época do ano, resfriamento, caprino

INTRODUCTION

In the last decades, research has focused on the improvement of biotechnology for animal breeding and methods of semen preservation for artificial insemination, embryo transfer and *in vitro* fertilization (Trummer *et al.*, 1998; Holt, 2000; Yoshida, 2000; Pauw *et al.*, 2003). In this regard, there are also specific approaches for defining the best method, interval and time of year for collection and preservation of semen samples (Amir *et al.*, 1986; Shamsuddin *et al.*, 2000; Campos *et al.*, 2003), as well as appropriate strategies for the dilution of those samples (Watson, 2000) and effective use of extenders (Singh *et al.* 1995; Gil *et al.*, 2003; Eiman AND Terada, 2004).

Recebido em 14 de outubro de 2011
Aceito em 28 de agosto de 2012
*Autor para correspondência (*corresponding author*)
E-mail: amoura@ufc.br

The reproductive performance of goat herds directly depends on the genetic potential of livestock, management and environment. The interrelationship of these factors determines the adaptation of the animals and their reproductive efficiency (Robertshaw, 1982). In temperate regions, animals with seasonal reproductive characteristics are influenced by a combination of photoperiod and temperature, while in tropical regions the environmental effect seems to be related to rain and its effect on the amount and quality of forage (Rege *et al*., 2000). However, other climate factors such as humidity and temperature changes can cause thermal discomfort, resulting in a decrease in food intake and interference with spermatogenesis and semen quality (Kunavongkrit *et al*., 2005).

Thus, this study was designed to evaluate the effect of seasons, defined as rainy and dry, in the cooling of caprine semen, sperm parameters (vigor, percentage of motile cells, degradation rate of sperm motility and morphology) and seminal plasma composition (calcium, phosphorus, magnesium, fructose, citric acid and total protein, PLA2 activity) in goats raised in the Northeast of Brazil.

MATERIALS AND METHODS

The experiment was conducted in the Northeast of Brazil, at 3º45' South and 38º32' West, 15.5m above sea level. The weather in this region is defined by Koppen as hot and humid (AW), with average temperature between 26 and 27ºC, maximum of 30ºC and minimum of 19ºC. The period of the study was year 2006, including a rainy season from March to May and a dry season from September to November. The former was characterized by an average rain fall of 345±123.4mm/year, humidity of 82.3±3.2% and temperature of 27±3.2ºC, varying from 24.4±0.4 to 30.3±0.4. In contrast, the dry season had an average rain fall of 5.4±4.3 mm/year, humidity of 69±1% and temperature of 27.6±0.1ºC, with amplitude from 25.2±0.3 to 31.1±0.2. The temperature and humidity index (THI) was determined as 75.1±1.2 for the rainy season, with a range from 73.6 to 76.8, and 75.4±1.5 for the dry season of the year, ranging from 73.3 to 77.5 (Silanikove, 2000).

We used 17 non-defined breed goats, with 26.2±5.4 months of age and 41.1±4.2kg. During the experiment, animals were raised in individual pens and fed Tifton hay (*Cynodon dactilum*) and concentrate (250g/head/day), following NRC (National…, 1981) recommendations. Semen was collected weekly for seminal plasma and biweekly for analysis of sperm parameters, using an artificial vagina model designed for sheep (Walmur®, Porto Alegre, RS, Brazil). In the first case, semen was centrifuged at 700g for 20 minutes (4°C) and the supernant seminal plasma stored at -18°C until use for biochemical analysis, as reported previously (Souza *et al*., 2010). In the case of samples collected biweekly, we recorded the ejaculated volume and diluted a 50-µL aliquot of semen in a phormol-saline solution (0.1% v/v) to determine sperm concentration using a spectrophotometer (Spectrophotometer SP M05 Bel® Photonics), according to Chemineau *et al*. (1991). The sperm concentration was measured and the semen samples were diluted in coconut water solution (ACP®) to obtain a final concentration of $2x10^8$ sperm/mL. From this solution, an aliquot was used to estimate the percentage of motile sperm and vigor, using a scale from 0 to 5 (Chemineau *et al*., 1991).

The media used to dilute semen samples was initially prepared as a stock solution, containing 50% of coconut water solution (ACP®) and 50 % citrate (2.5%), pH 6.2-6.8 and osmolarity at 300mOsmol/L. Immediately before mixing with semen samples, 2.5% of egg yolk was added to this stock solution (Cardozo *et al*, 2006). After the final dilution, semen samples were incubated at 4ºC and frozen for 48 hours. During this period, sperm vigor and percentage of motile cells was determined at 2, 24 and 48 hours. Additionally, we conducted the thermoresistance test (TRT), which consisted of incubating 250µL of semen at 38ºC, during 5 and 120 minutes, according to Chemineau *et al*. (1991). Semen aliquots were then collected, stained with bromophenol blue (1g bromophenol blue, 4g sodium citrate to 100mL distilled water) in glass slides and sperm morphology analyzed (200 cells/ejaculate; Derivaux, 1980). The degradation motility rate (DMR) was calculated at the end of the TRT, according to the formula: DMR = (sperm vigor at 5min. – sperm vigor at 120min.)/(sperm vigor at 5min.) x 100.

Considering the low volume of seminal plasma obtained from each ejaculation, all samples

collected in each month were pooled. Thus, this approach allowed us to have enough volume for all analysis of the seminal plasma components. Concentrations of Ca^{2+}, P, Mg and total protein were determined using commercial *kits* (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and those of citric acid and fructose, using Espermoteste® kits (InVitro Diagnostic S/A, Itabira-MG, Brazil), as described previously (Catunda *et al*., 2009). Phospholipase A_2 activity was quantified according to adaptations of the method published by De Haas *et al*. (1968). First, we prepared a solution containing one egg yolk in 50mL of distilled water. From this stock, 15mL was removed, mixed with 10mL of 0.03M sodium deoxycholate, 1mL of 0.6M $CaCl_2$ and distilled water q.s.q. in 100mL, pH 7.8-8.0. A 10mL aliquot of this final solution was added to 20µL of goat seminal plasma and pH was measured (PHS-3B, pHTEK). We analyzed the time required for a 0.03 pH unit decrease and then added 3µL of 0.11N NaOH. The methodology was carried out continuously during three minutes. After this, we averaged the time gaps between each time measured during the assay. This average was in turn multiplied by a correction factor to determine the total consumption of NaOH/minute/each 20µL aliquot of seminal plasma. For every sample, phospholipase A_2 activity was determined three times and the average of these repetitions was used for statistical analysis.

Variations in semen parameters and components of seminal plasma as related to season of the year were evaluated by analysis of variance and Tukey statistical test SAS (Statistical..., 2008). Variables defined as sperm motility and DMR were transformed in arcsen to fit normality requirements. Also, we used Pearson's method to estimate the correlations among sperm parameters and seminal plasma components (fructose, citric acid, total protein, $Ca^{2+,}$ P, Mg, and PLA_2 activity) within each season of the year SAS (Statistical..., 2008). Correlations were considered significant with p values <0.05.

RESULTS

In the case of fresh semen samples, the percentage of sperm with normal morphology was significantly higher (P<0.001) during the rainy season when compared to those collected in the dry season, but ejaculate volume, sperm motility, vigor and sperm concentrations did not differ between those periods of the year (P<0.05; Table 1).

Sperm parameters (mean ± S.E.M.) of goat semen samples collected during dry and rainy seasons in the northeast of Brazil

Season	Volume ejaculates (mL)	Percentage of motile cells	Vigor	Sperm concentration	Normal sperm morphology
Dry	0.9±0.1a	75.7±2.1a	3.4±0.1a	2.5x109±0.1a	82.8±2.5a*
Rainy	0.9±0.1a	77.9±1.9a	3.4±0.1a	2.5x109±0.1a	86.9±1.8b*

Values with different letters (a-b) in the same column differ significantly (P<0.05); (*P<0.0001).

When semen samples were subjected to cooling for 2, 24 and 48 hours, the percentage of motile cells and sperm vigor were always lower in the dry as compared to the wet season (P<0.05; Table 2). Moreover, the degradation motility rate (DMR) measured at 2, 24 and 48 hours of cooling time was 2, 1.8 and 1.6-fold higher in the dry than in the rainy season (P<0.05). Sperm morphology, determined in semen samples subjected to cooling, was different between dry and rainy seasons but not associated with cooling time regardless of the period of the year (Table 2).

Concentrations of Ca^{2+} remained without major changes throughout the study (P>0.05) and those of fructose, citric acid, P, Mg and total protein in the goat seminal plasma were consistently lower in the dry season compared to the rainy season (P<0.05). The greatest variations were associated with the concentrations of P and fructose, representing 26.8 and 31.3 % less in the dry when contrasted with the rainy season, respectively. Phospholipase A_2 activity was 32 % higher in the dry season when compared to the rainy period (P<0.05; Table 3).

Table 2. Sperm parameters (mean±S.E.M.) of goat semen samples collected during dry and rainy seasons and subjected to cooling at 4ºC during 48 hours

Hours after cooling	Percentage of motile cells		Vigor (0-5)		DMR (%)		Normal sperm morphology (%)	
	Dry	Raining	Dry	Raining	Dry	Raining	Dry	Raining
02	51.0±4.2aA	58.2±2.6aB	2.1±0.2aA	2.6±0.1aB	57.6±9,1aA*	28.2±6.2aB*	80.8±1.9aA	84.9±2.0aB
24	40.1±6.4bA	51.7±3.5bB	1.6±0.3bB	2.3±0.2bA	50.5±9.1abB*	28.2±7.3aB*	81.8±2.0aA	85.9±2.0aB
48	30.7±6.8cA	44.2±4.1cB	1.3±0.3cA	2.1±0.2cB	43.5±9.0bB*	27.1±7.4aB*	81.9±1.7aA	86.0±1.7aB

Values with different letters (a-b) in the same column and (A-B) in the same line differ significantly ($p < 0.05$); (*$P<0.0001$). DMR = degradation motility rate.

Table 3. Biochemical (mean ± S.E.M.) parameters of the goat seminal plasma collected during dry and rainy seasons in the northeast of Brazil

Season	Calcium (mg/dL)	Phosphorus (mg/dL)	Magnesium (mg/dL)	Total protein (g/dL)	Citric acid (mg/dL)	Fructose (mg/dL)	PLA_2 activity (U/mL)*
Dry	12.1±0.6a	9.7±0.9a	8.1±0.9a	5.2±0.4a	435.0±27.1a	502.6±51.0a	8.1±0.8a
Raining	12.3±0.5a	12.3±0.7b	8.6±1.0b	6.1±0.3b	495.4±30.0b	660.1±39.4b	6.2±0.5b

Values with different letters (a-b) in the same column differ significantly ($P<0.05$).
*U = µmol de fatty acid released per minute.

DISCUSSION

The present study describes the variations of goat semen criteria and certain components of seminal plasma associated with periods of the year characterized by pronounced differences in rain fall. The region where those animals were raised, the Northeast of Brazil, was at 3°45' of latitude, thus with no significant changes in day length and where seasons were defined mainly as dry and rainy. The former usually occurs from March to May, equivalent to autumn, and the later takes place from September through November, timely with the spring season in the Southern hemisphere.

Parameters such as volume of ejaculates, sperm concentration, percentage of motile cells and sperm vigor in fresh semen samples did not show significant differences related to periods of the year, but the number of sperm with normal morphology decreased in the dry season. However, when semen samples were subjected to cooling up to 48 hours, more specific and significant differences became evident between dry and wet seasons. In this case, the percentage of motile cells, sperm vigor and the number of morphologically normal cells were lower while DMR was higher in the dry season as compared to the wet period of the year.

The fact that sperm parameters in fresh semen did not differ between seasons cannot be associated with nutritional factors because all animals were confined and received the same diet during the experiment. However, there was a small increase in sperm pathology during the dry season, which can be due to the higher air temperatures reached during that period. Results similar to the ones presented here were found by Dias *et al*. (1995) and Vieira *et al*. (2008), who also reported significant increases in sperm pathology in semen of goats during the drought seasons in the Midwestern and Northeastern Brazil, respectively. As well established, heat stress triggers alterations in the seminiferous epithelium, also with deleterious effects on semen quality. Experimentally induced elevation of testicular temperature causes significant increases in sperm pathology (Lagerlof, 1938) and, with the rising temperature of the normal testis, as observed in cryptorchid animals (Moretti *et al*., 2007) or experimental-induced heat stressed testes (Moreira *et al*., 2001), spermatogenesis can be completely arrested. The severity of testicular degeneration depends on time and temperature of exposure, but even an increase of 1 or 2°C for eight hours can cause marked changes in the spermatogenesis (Entwistle, 1992).

In the present study, when semen samples were subjected to cooling at 4°C, there was a

significant decrease in the percentage of motile cells and vigor throughout the cooling time in both seasons. The cooling process can cause irreversible damage to sperm due to heat shock, which can be minimized by the slow cooling of diluted semen in a ratio of 0.05ºC/min to 4°C (Pickett and Amann, 1993) and by adding extenders containing phospholipids, such as milk or egg yolk (Amann and Pickett, 1987; Den Daas, 1992). However, the dilution of goat semen in extenders containing egg yolk can be deleterious to sperm cells. This occurs because the goat semen presents characteristics that differentiate it from other species, being the most important the presence of phospholipase A, secreted by the bulbourethral glands. This phopholipase is also called Eyce (Egg yolk coagulating enzyme) or BUSgp60 (bulb urethral gland secretion; LeBoeuf *et al*., 2000) and is responsible for the reduced viability of sperm cells that have been cooled or frozen in extenders containing egg yolk or milk, respectively (Corteell, 1981).

Considering the absence of significant differences related to sperm motility and vigor of semen collected before cooling, we also suggest that differences associated with the cooling time between the dry and rainy seasons relate to biochemical changes found in seminal plasma in the respective periods. In fact, we observed significant reductions in phosphorus, magnesium, citric acid, fructose and total proteins in the seminal plasma collected in the dry when compared to the wet season. Changes in biochemical components of the goat semen as related to seasons of the year were also observed by Roca *et al*. (1993) and Catunda *et al*. (2009) and it is well known that minerals are important for electrolytic balance and essential for the conservation of goat semen. These components also play a diverse range of roles on reproductive systems, such as regulation of intra and extracellular enzymes, membrane proteins, second messengers, receptors and energy metabolism (Smith and Akinbamijo, 2000).

Most extenders use the composition of egg yolk as a basic component, since the protection of spermatozoa against thermal shock is favored by phosphatidylcholines (lecithins), the lipoprotein yolk and milk casein (Dee Haas, 1992). However, the dilution of goat semen in extenders containing egg yolk can be deleterious to sperm cells. This fact can be observed when comparing the degradation motility rate (DMR) with two hours of cooling in the dry and rainy seasons. The significant increase of DMR in the dry season is probably related to increased phospholipase A2 activity present in seminal plasma, which induced a reduction in sperm motility and vigor in the same period. The negative influence exerted by the phospholipases present in seminal plasma persists even at temperatures of cooling or freezing. Thus, according Corteel (1974), there was a reduction in the number of live sperm directly proportional to storage time. The presence of phospholipase in goat seminal plasma, as previously mentioned, is unfavorable to its conservation, either when cooled or frozen.

CONCLUSION

Results obtained in the present study indicate that native goats of non-defined breed, raised in a tropical region of Brazil, show variations in the biochemical components of seminal plasma during the dry and rainy seasons. In fresh semen, sperm morphology was the only parameter meaningfully affected by season. However, when semen was subjected to cooling with extender containing egg yolk, sperm motility was negatively affected in the dry season of the year. This fact was coincident with higher phospholipase A_2 activity present in seminal plasma during the dry season, suggesting that this enzyme triggered the reduction in sperm viability.

REFERENCES

AMIR, D.; GACITUA, H.; RON, M.; LEHRER, A.R. Seasonal variation in semen characteristics and the fertility of Finn cross rams subjected to frequent ejaculation. *Anim. Reprod. Sci*., v.10, p.75-84, 1986.

AMANN, R.P.; PICKETT, B.W. Principles of cryopreservation of stallion spermatozoa. *J. Equine Vet. Sci.*, v.7, p.145-173, 1987.

CAMPOS, A.C.N.; NUNES, J.F.; MONTEIRO, A.W.U. *et al.* Conservation goat spermatozoa at 4ºC during the dry and rainy season in Northeastern Brazil. *Rev. Braz. Reprod. Anim.*, v.27, p.620-624, 2003.

CARDOZO, J.; FERNÁNDEZ-JUAN, M.; FORCADA, F. *et al.* Monthly variations in ovine seminal plasma proteins analyzed by two-dimensional polyacrylamide gel electrophoresis. *Theriogenology*, v.66, p.841-850, 2006.

CATUNDA, A.G.V.; CAMPOS, A.C.N.; PEREIRA, J.F. *et al.* Monthly variation in the concentrations of macroelements in the goat seminal plasma in humaid tropical climate. *Cienc. Anim. Bras*., v.10, p.1177-1185, 2009.

CHEMINEAU, P.; CAGNIÉ, Y.; GUÉRIN, Y. *et al.* Training manual on artificial insemination in sheep and goats. Food and Agriculture Organization of the United Nations (FAO). *FAO Animal Production and Health Paper*. p. 211-222, 1991.

CORTEEL, J.M. Collection, processing and artificial insemination of goat semen. In: GALL, C. (Ed). *Goat Production.* London: Academic Press, p.171-191, 1981.

CORTEEL, J.M. Viabilité de spermatozoides de bouc conservés et congelés avec ou sans leur plasma séminal: effet du glucose. *Annales de Biologie Animal*, Biochimie et Biophysie, v.14(4-B), p.741-745, 1974.

DE HAAS, G.H.; POSTEMA, N.M.; NIEUWENHUIZEN, W.; VAN DEENEN, L.L.M. Purification and properties of phospholipase A from porcine pancreas. *Biochim. Biophys. Acta*., v.159, p.103-117, 1968.

DEN DAAS, N. Laboratory assessment of semen characteristics. *Anim. Reprod. Sci.*, v.28, p.87-94, 1992.

DERIVAUX, J. *Reproduction of domestic animals*. Zaragoza, Ed. Acribia, p.446, 1980.

DIAS, M.J.D.; FERREIRA, R.N.F.; FILHO, O.D.B.; ORSINE, G.F. Effect of seasonal change on goat spermatic production. *Pesq. Agrop. Trop.*, v.25, p.53-62, 1995.

EIMAN, M.E.; TERADA, T.A. Effects of egg yolk during the freezing step of cryopreservation on the viability of goat spermatozoa. *Theriogenology*, v.62, p.1160-1172, 2004.

ENTWISTLE, K. Effects of heat stress on reproductive function in bulls. Bull Fertility, *Proccedings...*, p.57-63, 1992.

GIL, J.; LUNDEHEIM, N.; SODERQUIST, L.; RODRIGUEZ-MARTÍNEZ, H. Influence of extender, temperature, and addition of glycerol on post-thaw sperm parameters in ram semen. *Theriogenology,* v.59, p.1241-1255, 2003.

HOLT, W.V. Fundamental aspects of sperm cryobiology: the importance of species and individual differences. *Theriogenology,* v.53, p.47-58, 2000.

KUNAVONGKRIT, A.; SURIYASOMBOON, A.; LUNDEHEIM, N. *et al*. Management and sperm production of boars under differing environmental conditions. *Theriogenology*, v.63, p.657-667, 2005.

LAGERLOF, N. Infertility in male domestic animals. *Vet. Med*., v.33, p.550-561, 1938.

LEBOEUF, B.; RESTALL, B.; SALAMON, S. 2000. Production and storage of goat semen for artificial insemination. *Anim. Reprod. Sci.*, v.62, p.113-141, 1938.

MOREIRA, P.M.; MOURA, A.A.A.; ARAÚJO, A.A. Effects of scrotal insulation on testis size and semen criteria in Santa Inês hairy sheep raised in the state of Ceará, Northeast of Brazil. *Rev. Braz. Zootec*., v.30, p.1704-1711, 2001.

MORETTI, E.; CAIRANO, G.D.; CAPITANI, S. *et al.* Cryptorchidism and Semen Quality: A TEM and Molecular Study. *J. Androl*., v.28, p.194-199, 2007.

NATIONAL research council – NRC. Nutrient requirements of goats. Washington, D.C.: National Academy of Sciences, p.91, 1981.

PAUW, I.M.C.; SOOM, A.V.; MAES, D. *et al*. Effect of sperm coating on the survival and penetrating ability of in vitro stored bovine spermatozoa. *Theriogenology,* v.59, p.1109-1122, 2003.

PICKETT, B.W.; AMMAN, R.P. Cryopreservation of semen. In: MICKINNON, A.O.; VOSS, J.L. (Ed.). *Equine reproduction*. Philadelphia: Lea & Febiger, p.769-789, 1993.

REGE, J.E.O.; TOE, F.; MUKASA-MUGERWA, E. *et al.* Reproductive characteristics of Ethiopian highland sheep. II. Genetic parameters of semen characteristics and their relationships with testicular measurements in ram lambs. *Small Rumin. Res.*, v.37, p.173-187, 2000.

ROBERTSHAW, D. Concepts in animal adaptation: thermoregulation of the goats. In: INTERNATIONAL CONFERENCE ON GOAT PRODUCTION AND DISEASE, 3., 1982, Tucson. *Proceedings*…Seotsdale: Dairy Goat J., 1982. p.395-397.

ROCA, J.; MARTINEZ, E.; VÁSQUEZ, J.M. Seasonal variations in fructose and citric acid in seminal plasma of Murciana-Granadina goats. *Small Rumin. Res*., v.10, p.219-226, 1993.

STATISTICAL Analysis Sistem - SAS. SAS User's Guide: Statistics. Inst. Inc., Cary, NC, USA, 2008.

SHAMSUDDIN, M.; AMIRI, Y.; BHUIYAM, M.M.U. Characteristics of buck semen with regard to ejaculate numbers, collection intervals, diluents and preservation periods. *Reprod. Domest. Anim*., v.35, p.53-57, 2000.

SMITH, O.B., AKINBAMIJO, O.O. Micronutrients and reproduction in farm animals. *Anim. Reprod. Sci.*, v.60-61, p.549-560, 2000.

SILANIKOVE, N. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci.,* v.67, p.1-18, 2000.

SINGH, M.P.; SINHA, A.K.; SINGH, B.K. Effect of cryoprotectants on certain seminal attributes and on the fertility of buck spermatozoa. *Theriogenology*, v.43, p.1047-1053, 1995.

SOUZA, C.E.A.; ARAÚJO, A.A.; OLIVEIRA, J.T.A. *et al.* Reproductive development of Santa Inês rams during the first year of life: body and testis growth, testosterone concentrations, sperm parameters, age at puberty and seminal plasma proteins. *Reprod. Domest. Anim*., v.45, p.644-653, 2010.

TRUMMER, H.; TUCKER, K.; YOUNG, C. *et al.* Effect of storage temperature on perm cryopreservation. *Fertil. Steril*., v.70, p.1162-1164, 1998.

VIEIRA, R.J.; CARDOSO, F.T.S.; AZEVEDO, L.M. *et al.* Influence of scrotal morphology and season on the quality of goat sêmen raised in Piauí State. *Rev. Bras. Cienc. Agrar*., v.3, p.376-380, 2008.

WATSON, P.F. The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci*., v.60-61, p.481-492, 2000.

YOSHIDA, M. Conservation of sperms: current status and new trends. *Anim. Reprod. Sci.*, v.60-61, p.349-355, 2000.