



1D mapping of seminal plasma proteins in Anglo-Nubian goats

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Abstract

The lack of information about seasonal variation of seminal plasma proteins in goats from Northeast Brazil led us to investigate the protein band distribution throughout the year. The experiment lasted one year (from April 2006 to March 2007) in which 230 semen collections from five adult goats (*Capra hircus*) were carried out, at ages ranging from 18 to 22 months, weighing 42.0 ± 10.0 kg, belonging to Anglo Nubian breed. A pool of seminal plasma samples from all the animals in each month of the year was submitted to 1D electrophoresis SDS-PAGE, 12.5%. The gels were analyzed through Life Science Software from UVP, Inc[®]. The correlation between air humidity and total seminal plasma proteins was not significant ($r = -0.24$; $P > 0.05$), while a moderate and significant correlation was found between temperature and total protein ($r = 0.39$; $P < 0.001$). The semen volume and concentration showed statistical difference ($P < 0.05$) between months throughout the year, while the motility and vigor did not ($P > 0.05$). The analyzed gels showed a high frequency of molecular mass bands below 50 kDa throughout the year, followed by protein bands from 50 to 100 kDa. The major numbers of bands occurred in October and November, while May was the month that presented a smaller number of protein bands. The molecular bands of 14, 22, 24, 40, 55 and 66 kDa were present in all the months of the year. The band of 16 kDa was found in 41.7% of the months. May was the only month that did not show the 30 kDa. August and September did not present the bands of 34 and 35 kDa, while October showed both bands. The 45 kDa band was found in 75% of the months, being present in January, May, July, September and November. The 46 kDa band was identified in June and August, while the 47 kDa was identified in April. The 70 kDa band was identified from May to November (58.3%), the 72 kDa band was found in January, March and April (25%) and bands of 92 to 97 kDa were found from April to December (75%). These results showed a band distribution variation throughout the year in the Anglo Nubian goat breed in the semi-arid area of Brazil. It is concluded that medium rainfall or water scarcity in the dryer months seems to promote variation in semen volume and concentration and total protein distribution

throughout the year. As no month variation was observed in semen motility and vigor, and protein bands probably related to fertility showed an uniform distribution throughout the year, it is suggested that there is no fertility variation during the year in Anglo-Nubian goats the semi-arid region.

Keywords: electrophoresis, seasonal variation, semen, seminal plasma.

Introduction

Observations at Artificial Insemination Center located in Northeast Brazil suggest that in some periods of the year, male goats show semen freezability while in other periods, this ability is not observed (Martinez and Eloy, 2005). Even though this area is located close to equator line with a tropical climate and no variation of daylight throughout the year, there are factors, such as air humidity and food availability which in natural conditions may affect semen composition.

The study of seminal plasma components can be an alternative to evaluate the reproductive system function and semen quality in goats (Mendoza *et al.*, 1989; Pinheiro *et al.*, 1996a, b; Santos *et al.*, 1998).

The seminal plasma contains secretions from testicles, epididymal ducts and accessory sexual gland sources (Evans and Maxwell, 1987), from which the sperm acquire many proteins during the epididymal transit and at ejaculation, possibly affecting their fecundity capacity (Miller *et al.*, 1990; Yanagimachi, 1994; Maxwell and Johnson, 1999).

The seminal plasma function in goats acts on motility activation (Azerêdo *et al.*, 2001), survival of sperm submitted to freezing (Nunes, 1982), protection against plasma membrane lipid peroxidation (Schoneck *et al.*, 1996) and sperm capacitating (Maxwell and Johnson, 1999; La Falci *et al.*, 2002). According to Graham (1994), seminal plasma from rams and bulls affects epididymal sperm motility. However, the ability of sperm cells to face the damage during cryopreservation appears to reside in the sperm cells themselves. Martinez-Pastor *et al.* (2006) found better epididymal sperm motility in deer by adding untreated and denatured seminal plasma to semen before cryopreservation and after thawing. Nunes *et al.* (1982), working with goats in France, observed during the non-

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breeding season, in which seminal plasma contained bulbo-urethral but little or no vesicular secretions, the deleterious effect of the bulbo-urethral secretions on sperm survival and motility was significantly inhibited by the vesicular secretions.

The seminal plasma proteins are originally from plasma blood and partially synthesized and secreted by testicles (Kato *et al.*, 1985), epididymis (Turner and Reich, 1987) and vesicular gland (Manjunath *et al.*, 1994).

Many protein components from seminal plasma were related to fertility in male bovine (Killian *et al.*, 1993), swine (Flowers, 1998) horse (Brandon *et al.*, 1999) and buffalo (Harshan *et al.*, 2009); with freezability in bulls (Roncoletta *et al.*, 1997; 2000; Roncoletta, 1999; Asadpour *et al.*, 2007), semen viability in bulls and rams (Al-Somai *et al.*, 1994; Barrios *et al.*, 2000) and in rams they were related to sperm characteristics (Yue *et al.*, 2009).

In male goats researchers investigated seasonal variation on seminal plasma (La Falci *et al.*, 2002; Souza *et al.*, 2009) and identified and characterized the spermadhesin (Teixeira *et al.*, 2006) and proteins with affinity to heparin (HAPs; La Falci *et al.*, 2002). Also in goats, Villemure *et al.* (2003) found the Goat Seminal Protein (GSP-14kDa, GSP-15kDa, GSP-20kDa, and GSP-22kDa), homologous to Bovine Seminal Proteins (BSP).

The difference between the seminal plasma composition in different periods of the year has already been described in bovine by means of 1D electrophoresis (Rocha *et al.*, 1974; Martins Júnior *et al.*, 1995; Roncoletta *et al.*, 1997, 2000; Roncoletta, 1999), but in goats it has been briefly studied (La Falci *et al.*, 2002; Souza *et al.*, 2009).

The fecundity process needs an adequate composition of seminal plasma in order to allow the sperm metabolic process in natural breeding as well as

in semen freezability technology (Töpfer-Petersen *et al.*, 2005); so information concerning variation of seminal composition throughout the year is of relevant importance.

The objective of the present work was to map the seminal plasma protein distribution along the year in goats of Northeast Brazil by means of 1D electrophoresis and to correlate possible total protein variations with environmental factors. Seminal plasma parameters were also studied.

Materials and Methods

Animals

Five adult goats (*capra hircus*) from Anglo Nubian breed weighing 42.0 ± 10.0 kg, at ages between 18 and 22 months were used. The experiment was carried out at Embrapa Goats and Sheep located in Sobral – Ceará, Brazil. Geographically this area is positioned at 3°41'32" of South latitude, 40°20'53" of West longitude, 75 meters of altitude showing semi-arid climate with minimum and maximum mean temperature of 25 and 28°C, respectively; minimum and maximum mean air humidity of 57 and 81%, respectively and medium rainfall of 653 mm (Table 1; FUNCEME, 2008).

The animals were submitted to semi extensive management, receiving elephant grass (*Pennisetum purpureum* Schum.), leucaena hay (*Leucaena leucocephala* (Lam) de Wit.), concentrate composed of corn (*Zea Mays*.) and soybean bran (*Glycine max* (L.) Merr.), offered according to NRC (Nutrient, 1985) and mineral mixture. The ethical concerns were taken into consideration once the animals were placed in large and clean areas with access to covered installations. Animals were treated according to the rules of the Animal Welfare.

Table 1. Meteorological data from North area of Ceará State, Northeast Brazil, during the period from April 2006 to January 2007.

| Month | Medium rainfall (mm) | Medium temperature (°C) | Medium air humidity (%) |
|----------------|-------------------------|----------------------------|----------------------------|
| January - 2007 | 00 | 29.0 | 59.1 |
| February/07 | 260 | 26.0 | 78.0 |
| March/07 | 40 | 25.6 | 80.1 |
| April/06 | 360 | 25.3 | 83.9 |
| May/06 | 51 | 25.0 | 83.3 |
| June/06 | 00 | 24.9 | 77.4 |
| July/06 | 00 | 26.4 | 63.9 |
| August/06 | 00 | 27.5 | 57.1 |
| September/06 | 00 | 28.4 | 55.1 |
| October/06 | 00 | 28.7 | 54.3 |
| November/06 | 00 | 28.7 | 55.5 |
| December/06 | 00 | 28.6 | 58.8 |

Source: FUNCEME, 2008.

Semen evaluation

The following variables were analyzed throughout the year: volume, vigor, individual motility and concentration according to Brazilian College of Animal Reproduction (Manual, 1998).

The semen concentration was evaluated by using the spectrophotometer Spectronic 20 at dilution of 1:400 in saline solution.

Dosage of total proteins

Ejaculations were collected weekly by means of artificial vagina from April 2006 to March 2007, totaling 230 collections.

In order to analyze the total proteins, the semen was centrifuged at 10000 g for 30 minutes at 4°C, following the supernatant separation of sperm cells and kept in eppendorff tubes at -4°C until assayed. The total proteins were determined by the Bradford (1976) method based on the link of the Comassie Brilliant Blue G250 to proteins showing a blue color. The presence of proteins is observed through spectrophotometer PF-901 (Chemistry Analyzer Labsystems) using absorbance method at 595 wave length with bovine serum albumin (BSA) as standard.

ID electrophoresis

A 200 µg of protein aliquot from weekly seminal plasma samples of each animal was taken in each month compounding a pool, from all the animals in each month of the year. These materials were submitted to one dimensional electrophoresis (SDS PAGE) in gels of 10 x 8 cm with 12% of polyacrilamide concentration according to Hames (1981). Once the gels were ready to use, 10 µg of plasma sample with 20 µg of protein per each well were added to the gel plaque by using as standard the LMW electrophoresis calibration from Pharmacia Biotechnology which contains 94, 67, 43, 30, 20.1 and 14.4 kDa. The electrophoresis runner was made by using electrical current of 40 milieu ampere, electric tension of 170 volts and electrical potency of 7 watts for approximately 2 hours. The gel color was made by using the Comassie Brilliant Blue G-250 for approximately 2 hours and after that discolored in 30% methanol solution and 7% acetic acid in nanopure distilled water for 3 hours. Once the bands were visualized, the gel was submitted to a photodocumentation (Bio Doc-IT and Visidoc-IT Gel Documentation systems, UVP) and then analyzed by Doc-IT-LS® 6.0 software. In order to dry the gel, 50% methanol and 1% glycerol in nanopure distilled water were used for 10 minutes and after that the gel was placed between two cellophane sheets.

Statistical analyses

GLM from SAS, version 6, was used in order

to evaluate possible variations of seminal parameters and protein molecular mass throughout the year. Correlations between medium temperature and medium air humidity with total seminal plasma protein and correlations between the seminal plasma parameters (volume, concentration, motility, and vigor) were assessed. Statistical significance was accepted from $P < 5\%$.

Results

Significant difference ($P < 0.05$) was observed in total protein concentration within the months of the year. The medium concentration was $23.23 \pm 6.5 \mu\text{g}/\mu\text{l}$ ranging from 8.47 to $34.89 \mu\text{g}/\mu\text{l}$ throughout the year. The correlation ($r = -0.24$; $P > 0.05$) between air humidity and total seminal plasma proteins (Fig.1) was not significant, while a moderate and significant correlation was found ($r = 0.39$; $P > 0.001$) between temperature and total protein (Fig. 2).

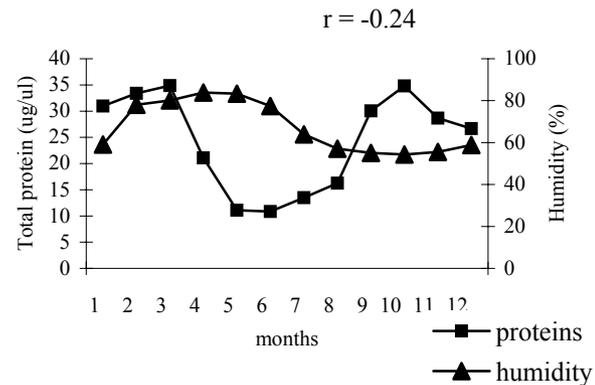


Figure 1. Correlation between total seminal plasma proteins (µg/µl) and medium air humidity (%) during the period from April 2006 to January 2007 in Anglo Nubian goats in the Northeast semi-arid region of Brazil.

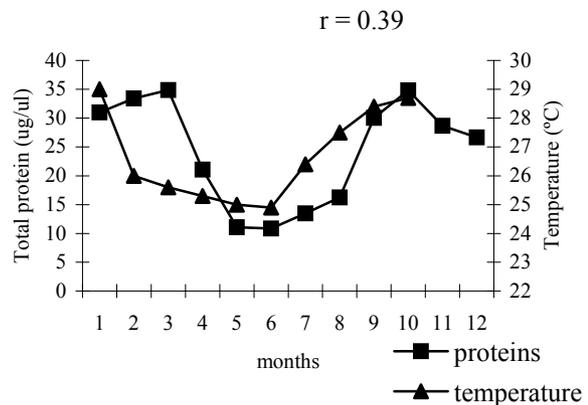


Figure 2. Correlation between total seminal plasma proteins (µg/µl) and medium temperature (°C) during the period from April 2006 to January 2007 in goats in the Northeast semi-arid region of Brazil.

The semen volume and concentration (Fig. 3) showed significant difference ($P < 0.05$) between the months throughout the year, while the motility and vigor did not ($P > 0.05$). The correlation

between volume and concentration was negative and significant ($r = -0.75$; $P < 0.05$) and between the motility and vigor was positive and significant ($r = 0.76$; $P < 0.05$).

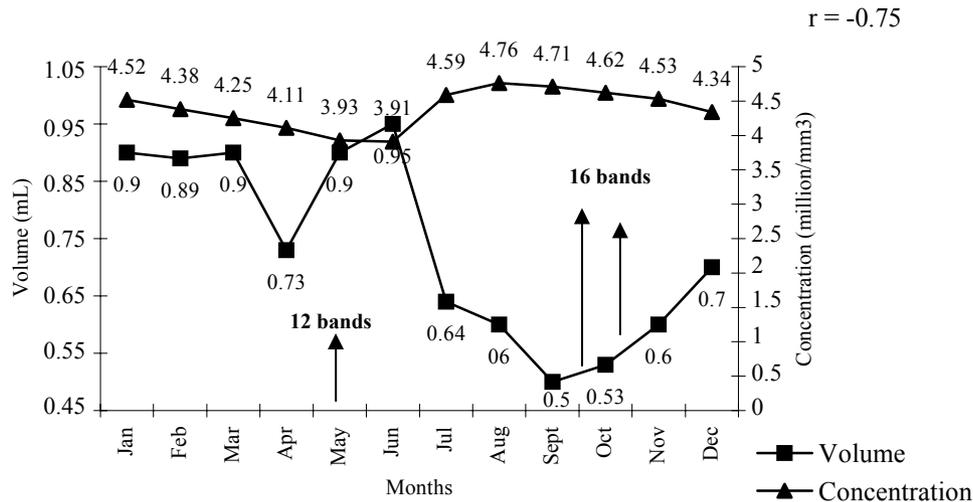


Figure 3. Correlation between semen volume (mL) and concentration ($\times 10^6 \text{ mm}^3$) throughout the year (April 2006 to January 2007) in goats in the Northeast semi-arid region of Brazil.

The analyzed gels (Fig. 4, 5) showed the following distribution: molecular band ranging from 14 to 100 kDa; high frequency of bands with molecular mass under 50 kDa, followed by molecular bands from

50 to 100 kDa. The higher number of bands occurred in October and November, in which 16 protein bands in gel samples were observed while May presented 12 bands, the month with the lowest number of bands.

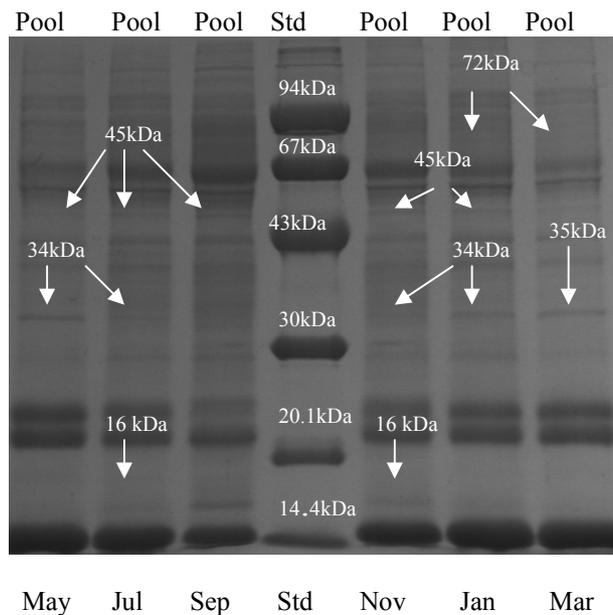


Figure 4. Analysis of proteins of pool from goat seminal plasma in the Northeast semi-arid region of Brazil by polyacrylamide gel electrophoresis SDS-PAGE, 12.5% in May, July, September, November, January and March. LMW ELECTROPHORESIS CALIBRATION from Pharmacia Biotech. 94: Phosphorylase b; Bovine serum albumin: 67; Ovalbumin: 43; Carbonic Anhydrase: 30; Soybean Trypsin Inhibitor: 20.1 and α Lactoalbumin: 14.4.

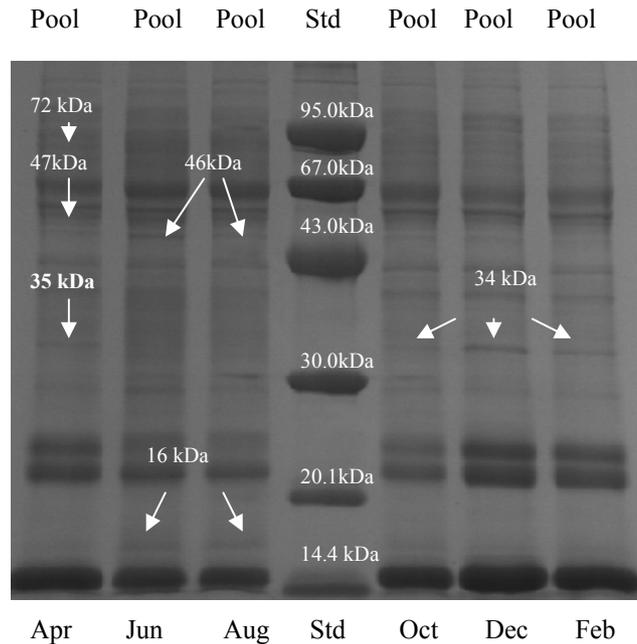


Figure 5. Analysis of proteins of pool from goat seminal plasma in the Northeast semi-arid region of Brazil by polyacrylamide gel electrophoresis SDS-PAGE, 12.5% in April, June, August, October, December and February. LMW ELECTROPHORESIS CALIBRATION from Pharmacia Biotech., 94: Phosphorylase b; Bovine serum albumin: 67; Ovalbumin: 43; Carbonic Anhydrase: 30; Soybean Trypsin Inhibitor: 20.1 and α Lactoalbumin: 14.4.

Table 2 shows bands of 14, 22, 24, 40, 55 and 66 kDa with uniform distribution throughout the year, while protein bands of 16, 30, 34, 35, 45, 46, 47, 70, 72 and 92 to 97 kDa presented no uniform distribution. The band of 16 kDa was found in 41.7% of the months. May was the only month that did not show the 30 kDa. August and September did not present bands of 34 and 35 kDa, while October showed both bands. The 45 kDa

band was found in 75% of the months, being present in January, May, July, September and November. The 46 kDa band was identified in June and August, while the 47 kDa was identified in April. The 70 kDa band was identified from May to November, the 72 kDa band was found in January, March and April, and bands of 92 to 97 kDa were observed from April to December.

Table 2. Distribution frequency (%) of the molecular protein bands throughout the year according to its molecular weight (kDa) in Anglo Nubian goats in the Northeast semi-arid region of Brazil.

| Gel proteins bands | Molecular weight (kDa) | Frequency (%) |
|--------------------|------------------------|---------------|
| 1 | 14.0 | 100 |
| 2 | 16.0 | 41.7 |
| 3 | 22.0 | 100 |
| 4 | 24.0 | 100 |
| 5 | 30.0 | 91.6 |
| 6 | 34.0 | 66.7 |
| 7 | 35.0 | 25.0 |
| 8 | 40.0 | 100 |
| 9 | 45.0 | 75.0 |
| 10 | 46.0 | 16.7 |
| 11 | 47.0 | 8.3 |
| 12 | 55.0 | 100 |
| 13 | 66.0 | 100 |
| 14 | 70.0 | 58.3 |
| 15 | 72.0 | 25.0 |
| 16 | 92.0 to 97.0 | 75.0 |



Discussion

In this study the semen volume and concentration showed statistical difference between the months throughout the year, while the motility and vigor did not. According to Chemineau (1986) and Hibbert *et al.* (1986) in tropical areas close to the equator line, where low luminosity variation is observed during the year, there is no seasonal effect on sperm production in male native bucks, in contrast with our findings. Differences among our results and previous reports could be attributed to other factors such as environment temperature (Nunes, 1988).

Silva *et al.* (2005), studying the effect of season on semen parameters in Anglo-Nubian x undefined crossbred buck-goats, submitted to a maintenance diet in the semi-arid area of Paraíba State, observed that vigor and motility were not affected while semen concentration presented statistical difference during the months of the year. This is in agreement with data from our study, although sperm concentration values found by these researchers were 2.9 and 1.8×10^6 spz/mm³ in the rainy and dry period, respectively, while our results were 4.1 and 4.6×10^6 spz/mm³ for the same periods. Conflicting results are possibly due to temperature and humidity variation and/or diet and breed differences. In this experiment medium temperature and medium air humidity were 25.2°C, 83.6% and 28.7°C, 54.9% for the rainy and dry period, respectively. While in the semi-arid region of Paraíba State, mean values of maximum and minimum temperatures and medium air humidity for the rainy (July to August) and dry (September to November) period were 33.3, 36.8°C, 51.9% and 22.7, 23.8°C, 45.0%, respectively. Finally, Zamiri and Heidari (2005) observed in Iran that semen quality and quantity of Rayini bucks were higher during the summer and early autumn.

In the current research semen motility and vigor did not show statistical difference throughout the year. Although progressive motility represents one of the characteristics necessary for sperm fertilization, the correlation between motility and the ability of the sperm cell to fertilize the oocyte is not yet fully clarified and findings are controversial among researchers (Sanchez-Partida *et al.*, 1999; Tardif *et al.*, 1999), and even the computerized analysis of motility and acrosome integrity do not present high correlation with fertility indices (Kjaestad *et al.*, 1993; Januskauskas and Zilinskas, 2002).

The average seminal plasma total proteins obtained throughout the year by Pinheiro *et al.* (1996a, b) in Moxotó and cross-breed Pardo Alpine goats ranged from 34.1 to 42.7 µg/µl, while this work presented a variation from 8.5 to 34.9 µg/µl. These different results can be explained by the nature of the Moxotó breed that is very well adapted to the semi-arid region. Souza *et al.* (2009), working with Alpine

American goats in Northeast Brazil observed lower total protein values during the high precipitation index period (data of the transition period is not shown). Azerêdo (2003) found a medium total protein value of 33.5 mg/ml in Saanen, Anglo-Nubian and Boer goats in the semi-arid area of the Northeast while average total proteins in this work was 23.2 µg/µl. This lower value obtained could be possibly due to an unusual dry period registered during this experiment, with complete absence of precipitation during seven months of the year affecting temperature, humidity and food availability (Murray *et al.*, 1990; La Falci *et al.*, 2002; Gebre, 2007).

According to Gebre (2007), in a tropical environment where the influence of photoperiod is minimal, nutrition appears to be the major modulator of sexual activity in small ruminants. Improving feed quality by increasing its protein content improved body weight, testicular size, semen characteristics and fertility (Abi-Saab *et al.*, 1997; Fernandez *et al.*, 2004) and increasing the energy intake improved testicular growth and sperm production (Braden *et al.*, 1974; Murray *et al.*, 1990).

In Southern Brazil, La Falci *et al.* (2002) found a range of molecular protein bands of Saanen goats in different seasons of the year and suggested that proteins from goat seminal plasma are under seasonal control and associated with sperm function during the breeding and nonbreeding seasons. In the present research, a range of protein bands was also observed even without the presence of seasonal daylength variation.

In the current study, high frequency of bands with molecular mass under 50 kDa was found throughout the year and five low mass molecular bands were observed from 14 to 30 kDa. According to Yue *et al.* (2009), most proteins in ram seminal plasma were below 30 kDa, but did not mention their distribution throughout the year. The 14, 22 and 24 kDa protein bands were present in 100% of the months while the 16 and 30 kDa bands were present in 41.7 and 91.6% of the months, respectively. Jobim *et al.* (2003), working with low mass molecular proteins from bovine, observed 12 proteins bands ranging from 15 to 26 kDa but did not relate them to the months of the year. The study of Bergeron *et al.* (2005), using SDS-PAGE analysis of alcohol-precipitated ram seminal proteins, also described the presence of about 25 proteins with molecular masses from 14 to 120 kDa and protein groups with a molecular mass of 15-16 kDa and 22-24 kDa were more predominant.

The months of October and November showed higher number of bands (16) coinciding with the higher semen concentration ($\sim 4.7 \times 10^6$ spz/mm³) and with the lower semen volume (~ 0.5 mL). Conversely, May presented lower number of bands (12) in correspondence with the higher volume (0.9 mL) and lower semen concentration (3.9×10^6 spz/mm³). So, water scarcity in the dryer months seems to promote



semen volume reduction and improve semen concentration and number of protein bands in seminal plasma producing higher quality semen, according to Zamiri and Heidari (2005).

A 14 kDa molecular band found in all the months in this experiment is probably related to a glycoprotein called spermadhesin present in semen or peripherally associated to sperm surface in several domestic animals, such as swine, bovine, horses and goats (Tedeschi *et al.*, 2000; Teixeira *et al.*, 2006). Also Chacur *et al.* (2006) observed a 13 kDa molecular weight protein in fertile bulls and bands of 18 and 20 kDa. In boars spermadhesins are a group of proteins found in seminal plasma, and they are major secretory products of the seminal vesicle epithelium. As these proteins are involved in the ability to bind glycoproteins to the zona pellucida, it has been suggested they have a role in sperm-egg interactions (Sanz *et al.*, 1993; Jonakova *et al.*, 1998; Ticha *et al.*, 1998).

The bands with molecular weight of 16 kDa were only observed in 41.7% of the months in the present experiment, showing the effect of environmental factors. Miller *et al.* (1990) described in bovine a group of 15-17-kDa seminal plasma heparin-binding proteins that were associated with the sperm membrane. Pérez-Pe *et al.* (2001), working with ram seminal plasma, observed that protein bands of 20, 21, 24, 36, and 67 kDa decreased in winter-spring and were even less intensely stained in the summer. According to these authors, the 21.5 kDa as well as the 20.5 kDa band were found during the non-breeding season. In this study, bands of 22 and 24 kDa were identified in 100% of the months of the year, suggesting no effect of the tropical climate on them.

Villemure *et al.* (2003) observed by analysis of pooled, dialyzed and lyophilized gelatin-agarose adsorbed protein fraction by SDS-PAGE from goat seminal plasma, the presence of four protein bands that were designated GSP-14 kDa, GSP-15 kDa, GSP-20 kDa and GSP-22 kDa (GSP, Goat Seminal Plasma Proteins). Pigozzo *et al.* (2008) found a 22 kDa band associated with improvement of sperm membrane integrity after thawing in swine. These authors observed that when this protein was present, the membrane integrity average was $47.9 \pm 6.9\%$ while in its absence was $32.0 \pm 2.0\%$, showing statistical difference. Bianchi *et al.* (2008) detected a 26.6 kDa band associated to a reduced (<55%) sperm membrane integrity in pigs after freezing and thawing.

The 30 kDa molecular mass band observed throughout the year (91.6%) in the current work was not identified in May, which is the transition period between the rainy and dry period. During May a lower number of protein bands was also observed. This band can be related to 30 kDa band, a group of similar proteins, collectively namely BSP-A1, BSPA2, BSP-A3, and BSP-30-kDa, called BSP proteins (Bovine Seminal Plasma Proteins) that are considered the major

proteins found in bovine seminal fluid. These proteins are produced by seminal vesicles and they bind to spermatozoa at ejaculation (Desnoyers and Manjunath, 1992). In bovine, some proteins have an effect on progressive motility induction together with the cAMP such as five proteins of 37.5 kDa (Shivaji *et al.*, 1990), proteins BSP-A1/-A2 and BSP-A3 with molecular mass of 15-17 kDa and 30 kDa, respectively, and BSP-30 with molecular mass of 28-30 kDa (Manjunath, 1984). Moura *et al.* (2007) also cited this 30 kDa band protein in bulls and suggested that interactions among several proteins in accessory sex gland fluid explained a significant proportion of the variation in fertility scores in the mature dairy sire. According to Desnoyers and Manjunath (1992) and Manjunath and Thérien (2002) the BSP protein binding sites on spermatozoa are phospholipids and they suggest that this specific interaction plays an important role in the sperm membrane lipid modification that occurs during capacitation and/or acrosome reaction. Manjunath and Thérien (2002) also observed that the presence of factors like heparin or HDL (high-density lipoproteins) in follicular or oviduct fluid would allow BSP proteins to act at the right time in the right place.

No literature citations on molecular mass bands of 34 and 35 kDa we observed in this experiment. These protein bands were absent in August and September in which the transition period of temperature between the rainy and dry period was apparent. It is possible to observe that the transition period identified during the year in this area affected the protein bands distribution.

Souza *et al.* (2009) found in American Alpine goats in Northeast Brazil, protein bands of 45 kDa during the high precipitation index period associated with a high percentage of intact acrosome (90.3%). Asadpour *et al.* (2007), working with seminal plasma in buffalo, described a protein of 45 kDa responsible for modifying sperm cells after thawing. Contrastingly, in the present study this band was observed during the dry and rainy periods. Probably different results are due to the lack of precipitation during seven months, from July to January, registered in this experiment, possibly affecting the quality and availability of food which in turn changes the seminal protein bands.

The present investigation showed a 55 kDa band in 100% of the months, suggesting that this protein is not affected by environmental factors in the semi-arid region in Northeast Brazil. This protein is an osteopontin of 55 kDa associated with fertility in bovine and it is considered a fertility marker present in the bovine seminal plasma (Killian *et al.*, 1993; Moura *et al.*, 2006), participating in sperm oocyte interaction during fertilization (Moura, 2005). According to Killian *et al.* (1993), Cancel (1999) and Gerena (2000) this protein modulates the cellular function receptors and modifies the sperm plasma membrane characteristics promoting the fertilization of the egg in addition to participating in the sperm capacitation.



A 66 kDa band observed in this study throughout the year is probably related to albumin, a protein involved in cholesterol extraction from plasma membrane (Yanagimachi, 1994; Flesch and Gadella, 2000) and important for the events proceeding fertilization.

By analyzing the number of bands observed through the software used in this investigation, it was possible to presume that it is responsible for isoform band detection which is usual in proteomic investigation, suggesting a possible involvement of them with semen quality.

The semen volume and concentration from Anglo Nubian goats in the Northeast semi-arid region of Brazil showed variation throughout the months of the year. Medium rainfall or water scarcity in the dryer months of the year seems to promote a decrease in semen volume and an increase of semen concentration and total protein distribution during the year. Semen motility and vigor showed no variation during the year. The seminal plasma protein bands from Anglo Nubian goats in the Northeast were affected by temperature throughout the year. As no month variation was observed in semen motility and vigor, and protein bands probably related to fertility showed a uniform distribution throughout the year, it is suggested that there is no fertility variation throughout the year in Anglo-Nubian goats in the semi-arid region.

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