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Artigo Completo

Seasonal variations in the seminal plasma composition of male goats

Abridged title: The seminal plasma composition of male goats

Júlio César Oliveira Dias*[1], Cristina Mattos Veloso[1], Madriano Christilis da Rocha Santo[1], Carlos Thiago Santos Alvim Mendes de Oliveira[1], Camila Oliveira Silveira[2], Erick Iglesias[1], Pedro Henrique. Furtado[3], Rita Flávia Miranda de Oliveira Donzele[1]

[1]Animal Science Department, Federal University of Viçosa - UFV, Viçosa, Minas Gerais – Brazil

[2]Veterinary Department, Federal University of Viçosa - UFV, Viçosa, Minas Gerais – Brazil

[3] Biochemistry Department, Federal University of Viçosa - UFV, Viçosa, Minas Gerais – Brazil

*Autor para correspondência: diasjuliovet@yahoo.com.br; Telefone: +55 31 9 9421-2987

I N F O A R T I G O

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R E S U M O

Este estudo avaliou as variações mensais proteicas e bioquímicas no plasma seminal de bodes da raça Alpina criados em clima tropical. A avaliação da motilidade progressiva de espermatozóides foi realizada no sêmen fresco e descongelado, e a avaliação da funcionalidade da membrana após a descongelação. Após 12 meses, o plasma seminal foi submetido a eletroforese em gel de SDS-poliacrilamida a 10 e 14%, em paralelo com as análises dos seus parâmetros bioquímicos. No gel de poliacrilamida a 10% foram identificadas 22 bandas proteicas (25-181 kDa), enquanto que no gel de 14%, foram identificadas 16 bandas proteicas (5,7-165 kDa). As frações de proteínas de 5,7 e 34,3-34,5 kDa mostraram um perfil que variou de acordo com a sazonalidade reprodutiva dos bodes, com o aumento da produção ocorrendo durante a estação reprodutiva. Na análise dos parâmetros bioquímicos, as concentrações de cálcio, fósforo, e colesterol também mostraram variação sazonal. No entanto, a análise da motilidade progressiva, bem como a funcionalidade de membrana não alteraram. Estes resultados indicam que alterações nos perfis proteico e bioquímico do plasma seminal durante o ano, não alterou a qualidade do sêmen fresco e descongelado de bodes Alpinos em clima tropical.

Keywords:
electrophoresis,
minerals,
proteins,
sperm

A B S T R A C T

This study evaluated the monthly changes in the seminal plasma protein and biochemical profiles of Alpine male goats that were bred in a tropical climate. The assessment of the progressive motility of spermatozoa was performed on fresh and thawed semen, the evaluation of the functionality of the membrane after thawing. After 12 months the seminal plasma was subjected to SDS-polyacrylamide gel electrophoresis at 10 and 14%, which paralleled the analysis of the biochemical parameters. In a 10% polyacrylamide gel, 22 protein bands (25-181 kDa) were identified, while in the 14% gel, 16 protein bands (5.7-165 kDa) were identified. The protein fractions of 5.7 and 34.3 to 34.5 kDa showed a profile that varied with the reproductive seasonality of male goats, with increased production occurring during the breeding season. In the analysis of the biochemical parameters, the concentrations of calcium, phosphorus, and cholesterol also showed seasonal variation. However, the analysis of the progressive motility, as well as the functionality of membrane no changes. These results indicate that changes in the protein and biochemical profiles of seminal plasma during the year did not alter the quality of fresh and thawed semen of Alpine male goats in a tropical climate.

1. Introduction

The seminal plasma is a complex mixture that is secreted by the testicles, epididymis, and accessory sexual glands and that can influence the spermatic cell in terms of morphology and freezability (Jobim et al., 2004), motility (Vison et al., 1996), capacitation, acrosome reaction (Roldan & Shi, 2007), which thus affect its fertilization ability and embryonic development (Monaco et al,. 2009). In addition to the actions that are directly linked to the regulation of spermatic function, the seminal plasma also helps with the protection, maintenance, and supply of metabolizable substrates to spermatozoids. This is possible due to its complex molecular composition, which is not yet well known (Yue et al., 2009) that vary among species (Rodger, 1975).

The most studied organic compounds in the seminal plasma are proteins, many of which have already been identified, isolated, and characterized (Jobim et al., 2009, Moura et al., 2011). The main category of proteins are those secreted by accessory sexual glands that link the surface (phospholipids) of the spermatozoids, modulate its physiological functions (Moura et al., 2011), and preserve the spermatic membrane during the seminal thawing process (Jobim et al., 2009). These proteins are collectively named BSP (Binder of Sperm Proteins) and have already been identified for cattle (Jobim et al., 2004), goats (Villemure et al., 2003), sheep (Jobim et al., 2005), horses (Brandon et al., 1999), and swine (Sanz et al., 1993). However, other proteins can also be involved in the protection of spermatic cells such as antioxidants (Schöneck et al., 1996), or related to the processes of motility (Baas et al. 1983), maturation, spermatic capacitation (Sylvester et al., 1991), and fertilization (Gonçalves et al., 2007).

Other compounds that can be highlighted in the seminal plasma are the minerals. They are considered to be the essential elements of semen because in addition to maintaining the electrolytic balance of the plasma, they participate in spermatic capacitation and maturation (Visconti et al., 1998) and several metabolic processes by acting as enzymatic cofactors (Catunda et al., 2009) or as activators/regulators of proteins (Visconti et al., 1998).

However, the structure of spermatozoids and their potential for fertilization depend not only on seminal proteins and minerals but also on the lipid composition in the plasmatic membrane and seminal plasma. The cholesterol is the highest lipid compound in the ejaculated semen of mammals, although it is found mainly in the plasmatic membranes of spermatic cells (Yeagle, 1993). During spermatic maturation, the epididymis synthetizes high amounts of cholesterol, which are transported to the plasmatic membranes to provide greater stability and protection against thermal and osmotic shock (Yanagimachi, 1994); after ejaculation in the reproductive tract of the female, it is removed from the plasmatic membrane to allow spermatic capacitation to occur (Visconti et al., 1998). Therefore, the spermatic morphology with regards to feasibility, maturation, functions, and potential of fertilization depend on the lipid composition of the plasmatic membrane and seminal plasma (Beer-Ljubic et al., 2009).

Despite the importance of seminal plasma in fertilization, few studies highlight the presence and possible functions of the components of seminal plasma of goats during the reproductive and non-reproductive seasons. These elements, mainly proteins, could be identified as markers of the reproductive capacity of males.

Knowledge of the biochemical components of semen can help in the identification of markers for the reproductive capacity of males, thus allowing for better evaluation of the fertility of breeding and the selection of semen donor goats. Moreover, as goats present with seasonal changes in their reproductive ability, studies can help to define the best season for semen preservation and may help to develop diagnostic and therapeutic techniques in the area of animal reproduction.

Thus, the objective of this study was to evaluate whether there are annual seasonal variations in the minerals calcium, phosphorus, and magnesium, or in the proteins and cholesterol in the seminal plasma of male Alpine goats that were raised in highland tropical climate conditions.

All procedures of handling were approved by the Ethics Committee for Animal Use of the Animal Science Department of the Federal University of Viçosa (process number 32/2013) and were performed according to the Ethics principles of animal experimentation as established by the Brazilian Animal Experimentation College and according to the current law.

2. Materials and methods

2.1 Locale and animals

The experiment was conducted during a twelve month period (March/2012 to February /2013) in the Goat section of the Animal Science Department of the Federal University of Viçosa (20°45'S, 42°52'W), Brazil, under natural lighting conditions. The climate of the region is the highland tropical type Cwa (dry winter and wet summer) based on the weather classification of Köeppen-Geiger, with an average temperature of 20.9°C and a precipitation index of 1,221 mm3/year. The amplitude of the photoperiod was 13 hours and 50 minutes of light for 10 hours and 50 minutes of darkness at the summer solstice, and 10 hours and 57 minutes of light and 13 hours and 3 minutes of darkness at the winter solstice; there was a difference of 2 hours and 13 minutes between the longest day and the shortest day in the experimental period (NO, 2013). In this region, the reproductive season is between March and July (autumn-winter) (Jainudeen et al., 2000; Thompson, 2004).

Four male Alpine Brown goats with an average age of 3.4 ± 1.9 years and fertility proven by natural mating were raised in individual pens. During all experimental periods, the same forage composed of corn silage, protein, and energy concentrate was provided, as well as free access to mineral salt and water to meet the nutritional requirements of the category according to the National Research Council – NRC (2007).

2.2 Semen evalation and seminal plasma preparation

The ejaculate from all animals were collected every fortnight by using an artificial vagina, always in the morning (7-8 h), while utilizing a female in estrus as a dummy; there were a total of 108 ejaculates by the end of the experimental period. The evaluation of semen progressive motility and the hypoosmotic test for evaluation of the membrane functionality were performed according to Yue et al. (2009) and Bittencourt et al. (2005) respectively.

After the evaluation, the semen was centrifuged at 1500 g for 10 minutes in an ambient temperature, after which the supernatant was centrifuged again under the same conditions. Then, samples of plasma from each animal were packed in plastic straws to freeze the semen (0.25 mL; IMV®) and were stored in liquid nitrogen (-196 °C). At the moment of the evaluation, the straws were thawed at 37°C for ten seconds and were poured into twelve 2-mL polyethylene tubes. Thus, each tube contained a pool of seminal plasma from all animals that were collected during the same month.

2.3 Determination of total protein, minerals, and sexual hormone concentrations in the seminal plasma

For the determination of the concentrations (mg/dL) of Ca+2, P+6, Mg+2, cholesterol, and total protein, the calorimetric method was implemented by using Invicto® commercial kits (In Vitro Diagnóstica, Brazil) and a

photometer Humalyzer Primus® (Human Diagnostics Worldwide, Germany) according to the specifications of the manufacturer.

2.4 Polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE was performed to determine the protein profile during the twelve month period and the molecular weight of the seminal plasma proteins of goats. The twelve samples of seminal plasma were submitted to SDS-PAGE according to the method previously described (Hames, 1981) while using polyacrylamide gels (SDS-PAGE) of 10% and 14%. The samples were misted with a sample buffer containing Tris base 0.1 M, SDS 4% (p/v), bromophenol blue 0.025% (p/v), glycerol 12%, and β-mercaptoethanol 10%, while approximately 30 µg of protein per sample was applied in the gel. Two gels were produced for each concentration (10% and 14%) and were run at the same time in an electrophoresis Mini-PROTEAN® system (BIO-RAD®, Richmond, USA); the first composite was made up of samples from months that encompass the reproductive season (March to August) and the second composite was composed of samples that encompass the non-reproductive season (September-February). A standard marker of molecular weight Broad Range® (BIO-RAD, Richmond, USA) composed of myosin (200 kDa), β-galactosidase (116.2 kDa), glycogen phosphorylase b (97.4 kDa), bovine serum albumin (BSA) (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa) were added in the second gel.

After the electrophoretic migration, the gels were immersed in a fixed solution containing glacial acetic acid 10% (v/v) and methanol 50% (v/v) for thirty minutes. After, they were stained in a solution containing ammonium sulfate 8%, phosphoric acid 0.8% (v/v), Coomassie Brilliant Blue G-250 0.08%, and methanol 30% (v/v) for 40 minutes before being bleached in a solution containing acetic acid 5% (v/v) for 72 hours.

2.5 Image acquisition and data analysis

The images of gels were analyzed to determine the molecular weight of seminal proteins while using the program Quantity One® (version 4.6, Bio-Rad, USA).

3. Results and discussion

The approximate molecular weights of seminal plasma proteins of Alpine goats in polyacrylamide gels of 10 and 14% are shown in Figure 1 and Table 1-2. The difference between the distribution and separation of the electrophoretic bands in gels A-B and gels C-D is due to the percentage of gel meshes. Thus, the polyacrylamide gels of 10% (images A-B) separate and highlight high molecular weight bands with greater distinction, while the gels of 14% (images C-D) separate those with lower molecular weights.

The results of the evaluation of seminal plasma show that a total of 22 protein bands were identified for the polyacrylamide gels of 10%, with molecular weights between 25.1 and 181.9 kDa, while 16 protein bands with molecular weights between 5.7 and 165.2 kDa were identified for gels of 14%. The proteins with a molecular weight below 30 kDa were slightly different from those verified by Jobim et al. (2005) and Yue et al. (2009) when they worked with sheep. However, data obtained in this study were close to those observed by Barrios et al. (2000) who, when working with sheep, verified 20 proteins in the seminal plasma, of which the majority were below 70 kDa. According to Yue et al. (2009), the differences that were found in the molecular weights can be influenced not only by the reproductive season, but also by the breed and the methods that were utilized to perform the collection and to prepare the seminal plasma.

The daylight (h) in the region of Viçosa/MG-Brazil (20°45'20''S and 42°52'40''W), the division of the sexual seasons among the months in this region, the values of progressive motility (0-100%) of fresh and thawed semen, and the values of the hypoosmotic test (%) of the thawed semen can be seen in Table 3.

When analyzing the images of Figure 1, we can observe that all identified protein fractions are observed during all experimental periods. However, the molecular weight band of 5.7 kDa, which can be seen in the images C and D (Figure 1), represented an irregularity in production during the year that was highlighted during the reproductive season (March-August; Table 2). The decapacitant proteins have a molecular mass around 5-10 kDa and avoid the spermatic capacitation that is caused by thawing the spermatozoids to prevent the increased concentration of calcium inside the spermatozoids (Flesch & Gadella, 2000). For Jobim et al. (2003), low molecular mass proteins (<15kDa) are involved in the maintenance of spermatic motility, and according to Ashworth et al. (1994), the protective effect of seminal plasma for sheep is determined by proteins that have a molecular weight from 5 to 10 kDa.

In this study, a protein band of 10.6-11.8 kDa was identified and can highlight the large concentration during all experimental periods according to Figure 1 (Image C-D). One of the proteins that is responsible for the protective action of spermatic cells is the acid protein of seminal fluid (aSFP; 11-12 kDa), which has an antioxidant action on the lipid peroxidation of the plasmatic membrane and thus preserves the feasibility of the spermatozoids (Schöneck et al., 1996, Jobim et al., 2003). Moreover, the aSFP has a role that protects the receptors of the spermatozoids, which in turn link the pellucid zone and should therefore be removed from the plasmatic membrane before the interaction of gametes (Dostalova et al., 1994).

The bands with molecular weights between 18.6-18.8 and 23.1-23.2 kDa (Images C and D) can correspond to the proteins that are linked to phospholipids; they are collectively named BSPs (Binder of Sperm Proteins), have a molecular weight between 15-17 kDa (BSP A1/A2), and are found in the seminal plasma of bulls at high freezability (Jobim et al., 2004). Villemure et al. (2003) verified four protein bands that were homologous to the BSPs in the semen of goats with molecular weights of 14, 15, 20, and 22 kDa and named them GSPs. The BSPs are synthesized by accessory glands and are linked to spermatozoids at the moment of ejaculation to later facilitate the capacitation through

Figure 1. Electrophoretic profile of proteins in the seminal plasma of goats over the course of twelve months in polyacrylamide gel - dodecyl-sodium sulfate 10 and 14 % (SDS-PAGE). A: gel 10% for the months of March, April, May, June, July, and August; B: gel 10% for the months of September, October, November, December. January, and February; C: gel 14% for the months of March, April, May, June, July, and August; D: gel 14% for the months of September, October, November, December. January, and February. At the left of each image, the proteins with a known molecular weight (PM; kDa) from the standard marker Broad Range® (BIO-RAD, Richmond, USA) are shown. The SDS-PAGE gels were strained with Coomassie Brilliant Blue R-250.

The following are the Broad Range® (BIO-RAD, Richmond, USA) molecular weight standards: myosin (200 KDa), β-galactosidase (116.2 KDa), glycogen phosphorylase b (97.4 KDa), bovine serum albumin (BSA) (66.2 KDa), ovalbumin (45 KDa), carbonic anhydrase (31 KDa), soybean trypsin inhibitor (21.5 KDa), and lysozyme (14.4 KDa).

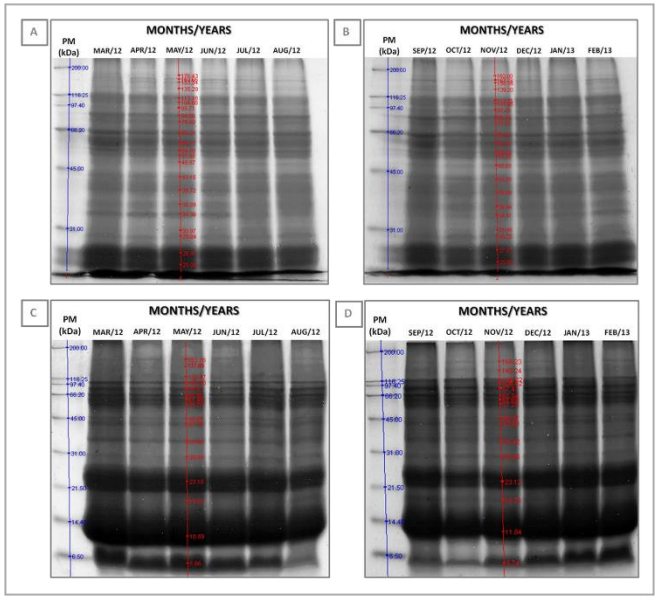

Table 1. Electrophoretic profile of the seminal plasma protein of goats over the course of twelve months in polyacrylamide gel – dodecyl-sodium sulfate 10% (SDS-PAGE).

SDS-PAGE gels 10 %													
PM (kDa)	MAR	APR	MAY	JUN	JUL	AUG	PM (kDa)	SEP	OCT	NOV	DEC	JAN	FEB
176.4	+	+	+	+	+	+	182.0	+	+	+	+	+	+
63.7	+	+	+	+	+	+	167.8	+	+	+	+	+	+
153.3	+	+	+	+	+	+	158.3	+	+	+	+	+	+
135.3	+	+	+	+	+	+	139.2	+	+	+	+	+	+
113.2	++	++	++	++	++	++	112.8	++	++	++	++	++	++
104.7	+	+	+	+	+	+	107.1	+	+	+	+	+	+
95.7	+	+	+	+	+	+	97.2	++	++	++	++	++	++
84.6	++	++	++	++	++	++	86.2	++	++	++	++	++	++
76.6	++	++	++	++	++	++	78.6	++	++	++	++	++	++
65.3	++	++	++	++	++	++	65.8	++	++	++	++	++	++
58.8	++	++	++	++	++	++	60.4	+++	+++	+++	+++	+++	+++
54.7	+	+	+	+	+	+	55.4	+	+	+	+	+	+
51.9	++	++	++	++	++	++	53.3	++	++	++	++	++	++
48.6	+	+	+	+	+	+	48.6	+	+	+	+	+	+
43.2	++	++	++	++	++	++	43.3	++	++	++	++	++	++
39.7	++	++	++	++	++	++	39.8	++	++	++	++	++	++
36.4	++	++	++	++	++	++	36.4	++	++	++	++	++	++
34.3	++	++	++	++	+	+	34.5	+	+	+	++	++	++
30.9	+	+	+	+	+	+	31.5	+	+	+	+	+	+
29.8	++	++	++	++	++	++	30.1	++	++	++	++	++	++
26.9	++++	++++	++++	++++	++++	++++	27.6	++++	++++	++++	++++	++++	++++
25.1	++++	++++	++++	++++	++++	++++	25.6	++++	++++	++++	++++	++++	++++

Table 2. Electrophoretic profile of the seminal plasma proteins of goats over the course of twelve months in polyacrylamide gel – dodecyl-sodium sulfate 14% (SDS-PAGE).

SDS-PAGE 14 % gels													
PM (kDa)	MAR	APR	MAY	JUN	JUL	AUG	PM (kDa)	SEP	OCT	NOV	DEC	JAN	FEB
162.8	+	+	+	+	+	+	165.2	+	+	+	+	+	+
137.9	+	+	+	+	+	+	140.2	+	+	+	+	+	+
120.6	+	+	+	+	+	+	119.2	+	+	+	+	+	+
104.2	+	+	+	+	+	+	106.6	+	+	+	+	+	+
84.6	+	+	+	+	+	+	87.4	+	+	+	+	+	+
65.6	+	+	+	+	+	+	65.9	+	+	+	+	+	+
61.5	+	+	+	+	+	+	61.7	+	+	+	+	+	+
56.8	+	+	+	+	+	+	57.3	+	+	+	+	+	+
45.1	+	+	+	+	+	+	46.1	+	+	+	+	+	+
42.7	+	+	+	+	+	+	43.0	+	+	+	+	+	+
35.4	+	+	+	+	+	+	35.4	+	+	+	+	+	+
29.9	+	+	+	+	+	+	29.9	+	+	+	+	+	+
23.2	++++	++++	++++	++++	++++	++++	23.1	++++	++++	++++	++++	++++	++++
18.6	+	+	+	+	+	+	18.8	+	+	+	+	+	+
10.6	++++	++++	++++	++++	++++	++++	11.8	++++	++++	++++	++++	++++	++++
5.7	+++	+++	+++	+++	+++	+	5.7	+	+	++	++	+++	+++

Table 3. Means and standard deviation of daylight (h) and progressive motility (0-100%) of fresh and frozen semen in the region of Viçosa/MG-Brazil (20°45'20''S and 42°52'40'' W) during the experimental period (Means ± SE).

Period	Daylight (h)	Sexual season *	Fresh semen	Thawed semen	
			Motility (0-100 %)	Motility (0-100 %)	Hypoosmotic (%)
March	12.23 ± 0.03 ^{f}	Reproductive	85.8 ± 1.49 ^{a}	45.4 ± 2.42 ^{a}	42.91 ± 4.61 ^{a}
April	11.63 ± 0.03 ^{h}	Reproductive	84.4 ± 1.75 ^{a}	45.0 ± 4.12 ^{a}	44.43 ± 5.80 ^{a}
May	11.15 ± 0.02 ^{j}	Reproductive	85.0 ± 1.89 ^{a}	46.3 ± 3.87 ^{a}	33.88 ± 2.81 ^{a}
June	10.91 ± 0.01 ^{l}	Reproductive	83.8 ± 1.57 ^{a}	40.6 ± 3.95 ^{a}	42.57 ± 5.21 ^{a}
July	11.05 ± 0.02 ^{k}	Reproductive	80.7 ± 2.02 ^{a}	41.4 ± 5.95 ^{a}	42.50 ± 4.41 ^{a}
August	11.46 ± 0.03 ^{i}	Reproductive	84.6 ± 1.79 ^{a}	43.2 ± 4.92 ^{a}	41.22 ± 5.95 ^{a}
September	12.02 ± 0.03 ^{g}	Non-reproductive	84.4 ± 2.74 ^{a}	41.9 ± 2.66 ^{a}	38.90 ± 5.91 ^{a}
October	12.62 ± 0.03 ^{e}	Non-reproductive	82.5 ± 2.83 ^{a}	43.8 ± 3.75 ^{a}	43.43 ± 3.98 ^{a}
November	13.14 ± 0.02 ^{c}	Non-reproductive	80.0 ± 2.11 ^{a}	35.6 ± 3.71 ^{a}	37.25 ± 5.09 ^{a}
December	13.39 ± 0.00 ^{a}	Non-reproductive	80.0 ± 2.50 ^{a}	36.3 ± 4.20 ^{a}	41.14 ± 6.62 ^{a}
January	13.26 ± 0.02 ^{b}	Non-reproductive	79.6 ± 2.08 ^{a}	41.7 ± 2.97 ^{a}	42.44 ± 5.31 ^{a}
February	12.80 ± 0.03 ^{d}	Non-reproductive	85.0 ± 2.50 ^{a}	48.1 ± 3.77 ^{a}	47.75 ± 5.50 ^{a}

Different letters in the same column differ among them by the Ryan-Einot-Gabriel-Welsh test (P<0.05);
*According to Jainudeen et al. (2004), Thompson (2004), and author observations.

Table 4. Monthly means of the biochemical parameters and protein profile of the seminal plasma of Alpine Brown goats over the course of twelve months.

Parameters	Reproductive season							Non-reproductive season						
	MAR	APR	MAY	JUN	JUL	AUG	Mean	SEP	OCT	NOV	DEC	JAN	FEB	Mean
Ca^{+2} (mg/dL)	14.1	15.1	15.0	15.4	14.2	10.1	14.0	11.8	10.9	10.7	11.5	13.4	14.2	12.1
P^{+6} (mg/dL)	9.0	6.8	7.8	7.3	9.3	7.4	7.9	6.9	11.4	11.3	8.5	9.1	9.3	9.4
Mg^{+2} (mg/dL)	5.3	5.2	5.3	5.3	5.5	5.5	5.4	5.4	5.4	5.4	5.4	5.3	5.4	5.4
CLT (mg/dL)	41.0	42.0	44.0	49.0	60.0	58.0	49	55.0	76.0	77.0	65.0	59.0	54.0	64
TP (g/dL)	4.2	4.3	4.1	4.1	3.8	2.8	3.9	2.9	4.2	3.9	3.7	4.1	3.7	3.8

CLT: cholesterol; TP: total proteins.

the removal of the cholesterol and phospholipids of the plasmatic membrane (Thérien et al., 1999). Yue et al. (2009) and Rueda et al. (2013) obtained a correlation (r= 0.47 and r= 0.64, respectively) of protein bands at 18.7 and 16.2 kDa in seminal plasma with spermatic feasibility. According to Jobim et al. (2009), the BSPs can protect the properties of the spermatic membrane during the cryopreservation process, thereby helping with the recovery of the plasmatic membrane permeability after being submitted to cold shock (Barrios et al. 2000).

Chacur & Machado Neto (2007), when working with cattle, verified a protein band of 26 kDa during the summer, although according to data presented in Figure 1 (Images A-B), the protein bands that were close to this value (25.1-26.9 kDa) were observed in the seminal plasma of goats and were present during all experimental periods. This band could be the prostaglandin D-synthase protein of the lipocalin class at 25-26 kDa, which was a low freezability indicator in cattle semen (Jobim et al., 2009). Yue et al. (2009) found correlations of r= - 0.535 and r= - 0.398 between a protein band of 25.37 kDa and the spermatic motility and pH of sheep respectively. The authors observed decreased protein values for the seminal pH, which will impair spermatic survival. However, according to some authors, the clusterin protein also has a molecular weight close to 25.35 kDa, and its effect would not impair spermatic cells (Pankhurst et al., 1998). This protein was responsible for lipid redistribution in the plasmatic membrane, and therefore was involved in the processes of spermatic maturation (Sylvester et al., 1991) and cryopreservation (Jobim et al., 2004).

The protein band of 34.3-34.5 kDa was equal to 5.7 kDa, which presented with an irregular production during the year while decreasing its concentration during part of the non-reproductive period (August to October) (Figure 1; images A-B). However, a complete absence of this band was not observed for any month, although a decrease in its concentration was observed and consequently, a lower value was observed from August to February (Table 2). The protein fractions of 5.7 and 34.3-34.5 kDa seem to be influenced by seasonality of the photoperiod, which can have a large influence on the seminal quality during the year. However, according to data of progressive motility for both fresh and thawed semen (Table 2), changes were not observed in this parameter (P>0.05) during the experimental period.

In the images A, B, C, and D of Figure 1, the presence of protein bands of 65.6 to 65.9 kDa can be observed, which can correspond to seminal albumin proteins that have a molecular weight of 66 kDa. Several actions are attributed to this protein in the semen such as stimulation of spermatic motility (Baas et al., 1983), antioxidants in the form of aSFP, and help with the spermatic capacitation via the BSPs (Thérien et al. 1999). The spermatic capacitation is an essential and necessary process that the spermatozoids must accomplish pass to fertilize the oocytes efficiently. Of the seminal plasma proteins that are related to the fertilization process, osteopontin (OPN; 55 kDa) is highlighted; in the image A-B of Figure 1, it could be the protein band at 54.7-55.4 kDa. It allows for an interaction between the spermatozoid and oocyte (Gonçalves et al., 2007), fertilization, and embryonic development (Moura et al., 2006, Monaco et al., 2009).

The loss of the integrity of the plasmatic membrane of the spermatozoid, mainly during the processes of cryopreservation and thawing, can decrease the spermatic

motility (Holt, 2000). In addition to this, the reproductive seasonality can also interfere with the synthesis and release of protein seminal plasma, which has the function of protection (Schöneck et al., 1996), stimulation (Baas et al., 1983), and is needed to help with spermatic functions (Gonçalves et al., 2007). According to Correa et al. (1997), motility and vigor are the main and the most common parameters that are utilized for the evaluation of sperm quality in order to predict seminal quality because they present a positive correlation with semen fertility (Correa et al., 1997). According to data from Table 3, the progressive motility of fresh and thawed semen did not show a difference ($P>0.05$) among months, nor did the hypoosmotic test after thawing, which evaluated the functionality of the plasmatic membrane. Thus, due to the seasonality that occurred, there were some changes in the protein profile of goats, although there was no record of losses in quality for both fresh and thawed semen (Table 3).

The results of the biochemical and protein profile parameters that were verified in the seminal plasma of Alpine goats during the twelve months are shown in Table 4.

The mineral with the greater concentration was calcium, followed by phosphorus and magnesium. In Table 4, a seasonal variation in calcium can be observed during the year, with greater concentrations during the reproductive season (March-August) and lower concentrations during anestrus (September-February). This greater concentration during the reproductive season of females can be due to several functions that calcium has on the reproductive physiology; for example, the activation of proteins that participate in the process of energy mobilization in the spermatic cells (Pozzan et al., 1994) and the capacitation and acrosome reaction of the spermatozoid are essential processes for fertilization (Visconti et al., 1998).

In this study, phosphorus presented with a greater average during anestrus (Table 4). A deficiency in phosphorus can lead to infertility because this mineral participates in the metabolism of carbohydrates, lipids, proteins, and nucleic acids (Bacila, 1980). Thus, the decrease in the phosphorus concentration during the reproductive period can be due to the large use of phosphorus in the metabolic processes during this period. Even magnesium, in addition to helping with the stability of the spermatic membrane (Pinheiro et al., 1996b), also acts as an enzymatic cofactor for several reactions related to energy metabolism and nucleic acid synthesis (Catunda et al., 2009); however, it was maintained in the seminal plasma throughout the year (Table 4).

Catunda et al. (2009) and Aguiar et al. (2013), when working with goats raised in a tropical climate, also verified greater concentrations of calcium in relation to phosphorus and magnesium, although differences were not observed among the reproductive season such as in this study, but between the wet and dry seasons. Pinheiro et al. (1996b) also suggested that the rainful index can influence the electrolytic balance in the semen of goats, such that rainy period correlated with a greater amount of pasture and a better quality of the parture. Even Kaya et al. (2002) said that the collection method (artificial vagina and electroejaculator) and the frequency of collection can influence the biochemical variations in the seminal plasma of goats and sheep. However, in this study, the feeding was balanced, controlled, and standardized during all experimental periods, and all seminal collections were performed with the same methodology by artificial vagina. Then, it can be inferred that changes in biochemical and protein profile parameters of the seminal plasma of Alpine goats twelve months during the year may have been, in part, due to other ambient factors such as ambient temperature, relative humidity, and especially photoperiod.

The decrease in the concentration of cholesterol in the seminal plasma (Table 4) during the reproductive season can also be due to the increased production and protection of spermatic cells that tend to be of higher quality in this period. The cholesterol maintains the stability of the membranes and is responsible for the resistance of the membrane against cold shock based on the existing cholesterol:phospholipid ratio. Thus, the concentration of cholesterol in the plasmatic membranes of spermatozoids could be greater than the seminal plasma, since some proteins that are present in the semen such as clusterin have the function of transporting lipids and can redistribute them in the plasmatic membrane (Pankhurst et al., 1998, Jobim et al., 2004)

The values of total protein in the seminal plasma during the reproductive season were not greater than those verified among the reproductive seasons, as recorded by Pinheiro et al. (1996a), which were different from the data presented by Aguiar et al. (2013). According to Table 3 and Figure 1 (Images C-D), the decreased presence of low molecular weight proteins in the seminal plasma (5.6-5.7 kDa) can be attributed to lower plasma protein concentrations during the months of August and September. However, as described previously, the changes in the protein profile of the seminal plasma of goats during the experimental period does not alter the quality of fresh and thawed semen (Table 2).

4. Conclusion

In the polyacrylamide gels of 10 and 14%, 22 and 16 protein bands were identified respectively in the seminal plasma, and only those with a molecular weight of 7.5 and 34.3-34.5 kDa presented with a seasonality and an increase in production during the reproductive season. The concentrations of calcium, phosphorus, and cholesterol also presented with seasonal variations during the experimental period. However, the changes in protein and the biochemical profile of seminal plasma did not impair the quality of fresh and thawed semen of Alpine goats raised in highland tropical climate conditions.

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