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Semen quality and concentration of soluble proteins in the seminal plasma of Alpine bucks

Qualidade seminal e concentração das proteínas solúveis do plasma seminal de bodes da raça Alpina

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RESUMO

Objetivou-se estudar a qualidade seminal por testes complementares e comparar com os aspectos físicos, morfológicos e bioquímicos do sêmen de bodes da raça Alpina. O experimento foi realizado na Universidade Federal de Viçosa, situado a 20º45' S de latitude e 42º51' WG de longitude, durante os meses de janeiro e fevereiro e foram utilizados 3 bodes da raça Alpina criados sob condições intensivas. O sêmen foi coletado pelo método da vagina artificial. Em todas as amostras de sêmen (45 ejaculados), depois das análises físicas e morfológicas do sêmen, o teste hiposmótico foi realizado. Em 24 ejaculados, procedeu-se ao teste de termorresistência e, em 21, foi determinada a concentração total de proteínas solúveis do plasma seminal. Os machos caprinos apresentaram diferenças nos aspectos físicos e morfológicos do sêmen, no teste hiposmótico e termorresistência, mas não apresentaram diferenças nas concentrações totais de proteínas solúveis do plasma seminal. Os resultados do teste de termorresistência e hiposmótico apresentaram correlação positiva (r = 0,60). Conclui-se de acordo com os nossos resultados que a concentração de proteínas solúveis do plasma seminal não pode ser utilizada como parâmetro para predizer a qualidade seminal de bodes da raça Alpina.

Palavras-chave: bioquímica do plasma seminal, caprinos, teste hiposmótico

SUMMARY

It was aimed to study the *in vitro* seminal quality analyzed by complementary tests and to compare them with physical, morphological and biochemical aspects of male goat semen of the Alpine breed. This experiment took place at the Federal University of Viçosa, situated at 20º45' S latitude and 42º51' W longitude, Southwest of Brazil. It was done during the summer months of January and February, and three adult male goats of the Alpine breed were used in intensive conditions. The semen was collected by artificial vagina method. In all semen samples (45 ejaculates), after the physical and morphological analysis, the hiposmotic test was done. In 24 ejaculates, it were done thermo-resistance test, and in 21 ejaculates it were determined the concentration of total soluble proteins in seminal plasma. The male goats presented difference in the semen physical and morphological aspects, in the hiposmotic test and thermo-resistance test, but they did not presented difference in total soluble proteins concentration in seminal plasma. Results of the slow thermo-resistance test and hiposmotic test were positively correlated (r = 0.60). It was concluded, according to our results, that the concentration of total soluble proteins in seminal plasma can not be used as a parameter to predict the seminal quality of Alpine bucks.

Keywords: goats, hiposmotic test, seminal plasma biochemistry

INTRODUCTION

Complementary semen tests and additional testing of sexual behavior, associated with the reproductive performance on a natural mating can predict the reproductive potential of males, allowing us to rationalize the use of males under natural mating conditions (RODRIGUEZ-MARTINEZ, 2005).

Sperm cells have a capacity of fertilization linked not only to physical and morphological aspects, but also to biochemical aspects of the semen. The seminal plasma is a complex mixture from epididymal and accessory glands fluids (MUIÑO-BLANCO et al., 2008). Soluble proteins develop many important functions in spermatozoa metabolism and fertilization process (MAXWELL & JOHNSON, 1999; GADELLA, 2008). La Falci et al. (2002) demonstrated that concentration of many proteins molecules is under seasonal control and associated with sperm function during breeding and nonbreeding seasons.

Hiposmotic test is used to evaluate the functional integrity of plasmatic spermatozoa membrane (REVELL & MRODE, 1994; ROTA et al., 2000). Biochemical activity of plasmatic membrane is very important in capacitation process, and can be used to predict the reproductive potential of males (REVELL & MRODE, 1994; ROTA et al., 2000; BRITO et al., 2003; BACINOGLU et al., 2008). The hiposmotic test can be used to evaluate goat semen (FONSECA et al., 2005; LEBOUEF et. al., 2006).

The isolated assessment from only one of these aspects is not effective in predicting male fertility, so the combined use of techniques can be an important tool to detect males with high and low fertility under natural mating conditions or in artificial insemination program (RODRIGUEZ-MARTINEZ, 2006). This work was carried out with the objective to study the *in vitro* seminal quality of Alpine breed bucks analyzed by complementary tests and to compare them with physical, morphological and biochemical aspects in semen.

MATERIALS AND METHODS

This experiment took place at the Federal University of Viçosa, Minas Gerais State, situated at 20º45' S latitude and 42º51' W longitude, Southwest of Brazil. The local average altitude is 752m above sea level with a dry cold winter and rainy hot summer climate (CWA climate), according to Koeppen classification.

Three adult male goats of the Alpine breed were used during the summer months of January and February. Animals were kept in individual pens and fed with corn silage and a concentrate diet twice a day, according to requirements. Water and mineral salt were available *ad libitum*. The semen was collected three times a week by the artificial vagina method and using a female in induced heat as dummy. In total, five ejaculates were collected. Immediately after collection, ejaculate volume, sperm concentration (hemacytometer method), mass movement, straight progressive motility, classification of sperm cells defects (CBRA, 1998) and hiposmotic test were done for all ejaculates. The spermatozoa defects were analyzed using a phase-contrast microscope at 1000x of magnification and it were counted 200 sperm cells per ejaculated. From twenty-four ejaculates, it were submitted the thermo-resistance test and, from twenty-one, were used to determine the total soluble proteins concentration in seminal plasma.

Hiposmotic swelling test was done according to Revell & Mrode (1994) as follow. A sample of 10µL of semen was mixed in 1mL hiposmotic solution (dehydrated sodium citrate and fructose solution with 100mOsm/kg) and incubated for one hour in a water bath at 37ºC. After incubation, 20µL of the solutions containing semen were evaluated using a phase-contrast microscope at 1000x of magnification. A total of 100 spermatozoa were counted per ejaculated. Spermatozoa were classified as reactive (coiled tail) or not reactive (straight tail) according to description used by Revell & Mrode (1994). Spermatozoa reactive rate was calculated subtracting the percentage of coiled tails after hiposmotic incubation by percentage of coiled tails found in morphological exam *in natura* semen (MELO & HENRY, 1999).

Slow thermo-resistance test was accomplished after dilution of the semen with solution of dehydrated sodium citrate, fructose, penicillin and streptomicin (1 semen: 4 solution). It was used a thin layer of mineral oil to avoid the influence of oxygen in the samples. It was analyzed the straight progressive spermatic motility and spermatic vigor at 0; 60; 120 and 180 minutes in water bath at 37ºC (VOGLER, 1991; SIQUEIRA et al., 2007).

The concentration of the total soluble proteins in seminal plasma was determined by spectrophotometric method using Coomassie Brilliant Blue reagent (BRADFORD, 1976). Ejaculate was diluted with physiologic solution (0.9%) in 1:9 (semen: solution) proportion and centrifuged at 700g force for 10 minutes at room temperature to obtain the seminal plasma. These samples were stored in straws (0.5mL) and frozen at -196ºC (liquid nitrogen). After thawed in water bath at 37ºC for 30 seconds, these samples were centrifuged at 150g for 5 minutes for complete separation of the seminal plasma. These samples were diluted again in 1:9 in physiologic solution (0.9%) to determine concentration of the total soluble proteins.

The Lilliefors test was used to evaluate normality of the data. The homogeneity of variance was studied, using the Cochran-Bartlett test. Means, standard deviations and variation coefficient were done for all studied variables. ANOVA was used to evaluate the animal effect under complementary tests (thermo-resistance and hiposmotic tests) and total soluble proteins concentration in seminal plasma. If there was effect when the Fisher´s test was used, it would be realized the Tukey´s test at 5% of significance to compare the averages. Pearson´s correlations were done between physical and morphological aspects and complementary tests used in semen.

RESULTS

The temperature averages and standard deviations were 29.5°C±2.08°C in January and 32.42±1.2°C in February, and 80% and 74% of relative humidity of air, respectively.

The male goats presented difference in the physical and morphological aspects ($p<0.05$) (Table 1 and 2), except for spermatic concentration ($P>0.05$) (Table 1). Animal 2 had the worst results on the physical aspects, but only his ejaculate volume was superior in relation to the others ($P<0.05$). Regarding semen morphological aspects, animal 1 showed the highest percentage of total spermatozoa defects (Table 2).

Table 1. Means and standard deviation of the physical aspects of the *in natura* semen of adult male goats of the Alpine breed

Goat	Volume	Motility	Vigor	Mass motility	Spzt 106/mL
1	1,05 ± 0,38[b]	88.33 ± 6.45[a]	3,83 ± 0,36[ab]	4.13 ± 0.39[b]	3498 ± 1253[a]
2	1,44 ± 0,42[a]	82.33 ± 5.93[b]	3,40 ± 0,47[b]	3.63 ± 0.48[c]	3460 ± 879[a]
3	0,84 ± 0,35[b]	91.33 ± 3.51[a]	4,03 ± 0,48[a]	4.80 ± 0.36[a]	3902 ± 1162[a]
Mean	1,11 ± 0,45	87.33 ± 6.53	3,75 ± 0,50	4.18 ± 0.63	3620 ± 1103
CV (%)	40,73	7.48	13,50	15.11	30,47

[a,b,c] = differents lower letters in the same column indicate difference (P<0.05) for the test of Tuckey at 5%. Volume = ejaculate volume; Motility = straight progressive spermatic motility (%); Vigor = spermatic vigor (0-5 points); Mass motility = spermatic mass movement (0-5 points); Spzt 10^6/mL = spermatic concentration in millions by mL; CV (%) = coefficient of variation.

Table 2. Means and standard deviation of the morphological aspects of the *in natura* semen of adult male goats of the Alpine breed

Goat	Major	Minor	Total	Tail defects
1	5.06 ± 1.63[a]	10.33 ± 7.95[a]	15.36 ± 8.42[a]	4.63 ± 1.57[a]
2	3.53 ± 1.44[b]	6.40 ± 4.44[ab]	9.93 ± 4.52[b]	2.56 ± 1.96[b]
3	3.00 ± 1.63[b]	4.63 ± 3.94[b]	7.63 ± 3.86[b]	2.26 ± 1.60[b]
Mean	3.86 ± 1.77	7.11 ± 6.09	10.97 ± 6.67	3.15 ± 1.99
CV (%)	45.91	85.64	60.83	63.09

[a,b,c]Differents lower letters in the same column indicate difference (P<0.05) for the test of Tuckey at 5%. Major = major spermatozoa defects (%); Minor = minor spermatozoa defects (%); Total = total spermatozoa defects (%); Tail = tail spermatozoa defects (%); CV (%) = coefficient of variation.

Difference was detected among male goats in complementary tests (hiposmotic test and thermo-resistance test), having animal 2 had the worst results (p<0.05).It was not detected difference among male goats in spermatic motility decrease after 3 hours of incubation and the seminal plasma total soluble proteins concentration (P>0.05) (Table 3).

Positive correlations between the percentage of reactive spermatozoa after incubation in hiposmotic solution and physical aspects were found (p<0.05), being r = 0.30 for straight progressive spermatic motility; r = 0.33 for mass motility; r = 0.31 for spermatic concentration; except for ejaculate volume (r = -0.47). Straight progressive spermatic motility after 3 hours of incubation (thermo-resistance test) and hiposmotic test results were positively correlated (r = 0.60).

Table 3. Means and standard deviation of the percentage of reactive spermatozoa after hiposmotic test, thermo-resistance test and seminal plasma total soluble proteins concentration of adult Alpine breed male goats

Goat	Hiposmotic test	Thermo-resistance test	↓ Motility	Protein concentration g/dL
1	25.9 ± 9.8[a]	70 ± 7.07[a]	22 ± 7.5[a]	1.87 ± 0.83[a]
2	16.8 ± 5.7[b]	58 ± 5.7[b]	28 ± 6.7[a]	2.72 ± 1.75[a]
3	28.1 ± 7.62[a]	70 ± 7.9[a]	22 ± 8.3[a]	2,77 ± 1.15[a]
Mean	23.8 ± 9.1	66 ± 8.7	24 ± 7.6	2.45 ± 1.3
CV (%)	38.4	13.18	31.5	53.20

[a,b,c] = different lower cases in the same column indicate difference (P<0.05) for the test of Tuckey at 5%. Hiposmotic test = percentage of reactive spermatozoa after incubation in hiposmotic solution; Thermo-resistance test = straight progressive spermatic motility after 3ª hour of incubation (%); ↓ Motility = spermatic motility decrease after thermo-resistance test; Protein concentration g/dL = seminal plasma total soluble proteins concentration (g/dL); CV (%) = coefficient of variation.

DISCUSSION

Volume and straight progressive sperm motility averages in raw semen were higher than those observed by Bittencourt et al. (2006), which had 0,57mL and 62.8% and 0.57mL for straight progressive spermatic motility and semen volume, respectively. Spermatic concentration in millions (3410 ± 1060), major spermatic defects (2.36 ± 1.1%), minor spermatic defects (9.79 ± 3.2%) and total spermatic defects (12.14 ± 4.1) were very similar to this experiment.

The average result obtained in this experiment for hiposmotic test was lower than described by Santos et al. (2006). These authors worked during the spring season in the same herd, which had 57.2±15 of reactive sperm cells after hiposmotic incubation. The epididymal epithelium is dependent on high androgenic concentrations (MARENGO, 2008). In this case heat stress probably influenced the seminal quality, by altering spermatozoa maturation membrane in epididymis and consequently reducing the number of reactive spermatozoa after hiposmotic incubation.

This study showed high decrease in straight progressive spermatic motility after 3 hours of incubation in thermo-resistance test (Table 3). These results showed a decline in semen quality, without any visible changes in physical and morphological semen aspects, because physical and morphological aspects results were normal (Table 1 e 2). Probably it happened because the inadequate plasma membrane maturation in the epididymis decreased the viability of the sperm plasma membrane (MARENGO, 2008).

Soluble proteins concentration in seminal plasma determined in this experiment was lower than in works by other authors. Santos et al. (1998) also achieved higher means than our experiment, 5.14g/dL for crossbred bucks. But the method used for determining the protein concentration by those authors was not the same, with different sensitivity from that used in our experiment (SCOPE, 1987). De Souza et al. (2009) also used 3 males goats of Alpine breed and the Bradford protocol for total soluble proteins concentration determination and found 1,47g/dL and 1,03g/dL in high and low precipitation index periods, respectively. These results

were similar to the present experiment. In addition, these experiments were realized in Northeast of Brazil, a region near the equator line with different climate conditions. According to our results, the concentration of total soluble proteins in seminal plasma showed no relationship with the complementary tests on raw semen.

Considering together the hiposmotic test results and total soluble proteins concentration in seminal plasma, there was a decline in our results, which was expected, once the animals were outside the reproductive season during humid hot summer. After this experiment, these animals were submitted to controlled mating, and there were not detected differences among animals about hiposmotic test, concentration of total soluble proteins and pregnancy rate (MARTINS et. al., 2006). According these results, it is possible to conclude that total soluble proteins concentration in seminal plasma can not be used as a parameter to predict the seminal quality of Alpine bucks.

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REFERENCES

BACINOGLU, S.; TAS, M.; CIRIT, U.; OZDAS, B.O.; AK, K. The potential fertility estimation capacity of the hypoosmotic swelling test, the thermal stress test and a modified cervical mucus penetration test in the bovine. **Animal Reproduction Science**, v.104, n.1, p. 38-46, 2008. [Links].

BITTENCOURT, R.F.; RIBEIRO FILHO, A.L.; ALVES, S.G.G.; BISCARDE, C.E.; VASCONCELOS, M.F.; OBA, E.O. The effect of equilibration time on the quality of cryopreserved goat semen **Revista Brasileira de Saúde e Produção Animal**, v.7, n.1, p.27-37, 2006. [Links].

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Animal Biochemistry**, v.72, n.1-2, p.248-254, 1976. [Links].

BRITO, L.F.C.; BARTH, A.D.; BILODEAU-GOESEELS, PANICH, S.P.L., KASTELIC, J.P. Comparison of methods to evaluate the plasmalemma of bovine sperm and their relationship with in vitro fertilization rate. **Theriogenology**, v.60, n.8, p.1539–1551, 2003. [Links].

COLÉGIO BRASILEIRO DE REPRODUÇÃO ANIMAL - CBRA. **Manual para exame andrológico e avaliação de sêmen animal**. 2.ed. Belo Horizonte, 1998. 49p. [Links].

FONSECA, J.F.; TORRES, C.A.A.; MAFFILI, V.V.; BORGES, A.M.; SANTOS, A.; D.F.; RODRIGUES, M.T.; OLIVEIRA, R.F.M. The hypoosmotic swelling test in fresh goat spermatozoa. **Animal Reproduction**, v.2, n.2, p.139-144, 2005. [Links].

GADELLA, B.M. The assembly of a zona pellucida binding protein complex in sperm. **Reproduction in Domestic Animals**, v. 43, n.5, p. 12-19, 2008. [Links].

LA FALCI, V.S.N.; TORTORELLA, H.; RODRIGUES, J.L.; BRANDELLI, A. Seasonal variation of goat seminal plasma proteins. **Theriogenology,** v.57, n.3, p.1035-1048, 2002. [Links].

LEBOUEF, B.; LE VERN, Y.; FURSTOSS, V.; KERBOEUF, D.; GUILLOUET, P.; MAGISTRINI, M. Response of goat sperm to hypoosmotic steps modeled probit analysis. **Animal Reprodution Science**, v.36, n. 3-4, p. 65-274, 2006. [Links].

MARENGO, S.R. Maturing the sperm: Unique mechanmisms for modifying integral proteins in teh sperm plasma membrane. **Animal Reproduction Science**, v.105, n.1-2, p.52-63, 2008. [Links].

MARTINS, L.F.; PEREIRA, M.C.B.; GUIMARÃES J. D.; DA COSTA, E. P.; SILVEIRA, T.S.; TORRES, C.A.A.; RODRIGUES, M.T.; BRAZ, V.B. Reproductive performance, soluble proteins of the seminal plasm and hypoosmotic test in male goats of the Alpine breed under controlled mating. **Revista Brasileira de Zootecnia**, v.35, n.4, p.1651-1657, 2006. [Links].

MAXWELL, W.M.C.; JOHNSON, L.A. Physiology of spermatozoa at high dilution rates: The influence of seminal plasma. **Theriogenology**, v.52, n.8, p.1353-1362, 1999. [Links].

MELO, M.I.V.; HENRY, M. Hyposmotic swelling test in equine semen evaluation. **Arquivo Brasileiro de Veterinária e Zootecnia,** v.51, n.1, p.71-78, 1999. [Links].

MUIÑO-BLANCO, T.; PÉREZ-PÉ, R.; PÉREZ-CEBRIÁN, J.A. Seminal plasma proteins and sperm resistance to stress. **Reproduction in Domestic Animals**, v. 43, n.4, p.18-31, 2008. [Links].

REVELL, S.G.; MRODE, R.A. An osmotic resistance test for bovine semen. **Animal Reproduction Science**, v.36, n.1, p.77-86, 1994. [Links].

RODRIGUEZ-MARTINEZ, H. Can we increase the estimative value of semen assessment? **Reproduction in domestic animals**, v.41, n.2, p.2-10, 2006. [Links].

RODRIGUEZ-MARTINEZ, H. Methods for sperm evaluation and their relationship to fertility. In: CONGRESSO BRASILEIRO DE REPRODUÇÃO ANIMAL, 2005, Goiânia. **Anais**... Goiânia: CBRA, 2005. p.1-9. [Links].

ROTA, A.; PENZO, N.; VINCENTI, L.; MANTOVANI, R. Hypoosmotic swelling (HOS) as a screening assay for testing in vitro fertility of bovine spermatozoa. **Theriogenology**, v.53, n.7, p.1415-1420, 2000. [Links].

SANTOS, A.D.F.; TORRES, C.A.A.; FONSECA, J.F.; BORGES, A.M.; GUIMARÃES, J.D.; COSTA, E.P.; ROVAY, H. Use of complementary tests to evaluate the freezing ability of semen from goats under artificial photoperiod exposure. **Revista Brasileira de Zootecnia**, v.35, n.5, p.1934-1942, 2006. [Links].

SANTOS, D.O.; AZEVEDO, H.C.; SALLES, H.O.; PINHEIRO, R.R Effect of scrotal insulation on goats seminal plasma constituents. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.50, n.3, p.283-286, 1998. [Links].
SCOPE, R.R **Protein purification**: principles and practice. 2.ed New York: Springer-Verlag, 1987. p.276-283. [Links].

SIQUEIRA, J.B.; GUIMARÃES, J.D.; COSTA, E.P.; MARC HENRY, TORRE, C.A.A.; SILVA, M.V.; G.B.; SILVEIRA, T.S. Relationship between conception rates obtained by using bovine frozen semen and *in vitro* spermatic evaluation. **Revista Brasileira de Zootecnia**, v.36, n.2, p.387-395, 2007. [Links].

SOUZA, A.F.; LEITÃO, M.C.G.; BATISTA, A.M.; PORTO, A.L.F.; LIMA FILHO, J.L.; GUERRA, M.M.P. Proteins of goat seminal plasma related with precipitation index and semen quality. **Ciência Rural**, v.39, n.4, p.1155-1161, 2009. [Links].

VOGLER, C.J.; SAACKE, R.G.; BAME, J.H.; DEJARNETTE, J.M.; McGILLIARD, M.L. Effect of scrotal insulation on viability of cryopreserved bovine semen. **Journal of Dairy Science**, v.74, n.11, p.3827-3835, 1991. [Links].

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