NOTA BREVE

EFFECT OF INITIAL SEMINAL PLASMA FRUCTOSE CONCENTRATION ON GOAT SEMEN STORAGE AT 5 ^{O}C

EFEITO DA CONCENTRAÇÃO INICIAL DE FRUTOSE SOBRE A CONSERVAÇÃO A 5 °C DO SÊMEN CAPRINO

Matos-Brito, B.G.[1]; Lima, I.C.S.[1]; Pereira, J.F.[1]; Barboza, F.M.[1]; Linard, M.A.B.[1]; Aguiar, G.V.[2]; Catunda, A.G.V.[1]; Moura, A.A.A.[1]; Nunes, J.F.[2] and Campos, A.C.N.[1]*

[1]Department of Animal Science. UFC. Fortaleza, Ceara. Brazil. *acncampos11@gmail.com
[2]Faculty of Veterinary. State University Ceara. Fortaleza, Ceara. Brazil.

ADDITIONAL KEYWORDS

Citrate-egg yolk. Industrialized coconut water-egg yolk. Phospholipase A_2. TRIS-egg yolk.

PALAVRAS CHAVE ADICIONAIS

Água de coco industrializada-gema de ovo. Citrato-gema de ovo. Fosfolipase A_2. TRIS-gema de ovo.

SUMMARY

Twenty-four goat semen samples were collected and divided into four aliquots, diluted with the citrate-egg yolk (CY), TRIS-egg yolk (TY) or industrialized coconut water with egg yolk (ICW-Y) extenders. The fourth aliquot was centrifuged to analyze fructose concentration and PLA_2 activity on SP. The semen was stored at 5 ^{o}C and evaluated at times fresh, 2, 24 and 48 h, in each time was evaluated the vigor, sperm motility and total morphological alterations. The animals were divided into two groups: group I-fructose concentration >710 mg/dL and group II-fructose concentration <480 mg/dL. The ICW-Y extender presented inferior results. The effect of the group was observed only in the ICW-Y. CY and TY worked similarly in the two groups. No significant difference in the activity of PLA_2 was found between the groups. However, ICW-Y should not be used for the storage of goat semen.

RESUMO

Foram coletadas 24 amostras de sêmen caprino. Cada ejaculado foi dividido em 4 alíquotas, e foram diluídas em citrato-gema de ovo (CG), TRIS-gema de ovo (TG) e água de coco industrializada-gema de ovo (ACI-G), a quarta, foi centrifugada para determinação da concentração de frutose e atividade da FLA_2 no PS. O sêmen foi conservado a 5 ºC e avaliado a fresco, 2, 24 e 48 h, em cada tempo foi avaliado o vigor, motilidade e alterações morfológicas. Os reprodutores foram divididos em dois grupos: grupo I-concentração de frutose >710 mg/dL e o grupo II-concentração de frutose <480 mg/dL. O sêmen diluído em ACI-G apresentou resultados inferiores. O efeito de grupo foi observado apenas no sêmen diluído em ACI-G. CG e TG funcionaram de forma similar nos dois grupos. Nenhuma diferença na atividade da FLA_2 foi encontrada entre os grupos. Por outro lado, a ACI-G não deve ser utilizada para a conservação do sêmen caprino.

INTRODUCTION

Many studies have been carried out in an attempt to improve the fertilizing capacity of cooled or frozen goat semen that still displays unsatisfactory results when used *in vivo* (Leboeuf *et al.*, 2000). Therefore, several options have been proposed for the formulation of extenders to conserve the semen at 5 °C. One of the natural extenders used is whole cow milk (Evans and Maxwell, 1990). However, the dilution of goat semen with these extenders may be harmful to the spermatozoa, due to the PLA_2 enzyme present in the SP. PLA_2 are deleterious to sperm (Nunes *et al.*, 1982). The use of coconut water has simplified the technology

Recibido: 12-7-11. Aceptado: 26-6-12.

of storing liquid goat semen at 5 °C, thus avoiding the washing of SP and consequently the PLA_2 enzyme, because this extender is poor in phospholipids (Nunes, 1998). Another important aspect is that fructose concentrations ranging from 249.11 (Roca *et al.*, 1993) to 1,420 mg/dL (Barakat *et al.*, 1972), and supplies energy to the sperm. The objective of this study was to verify the quality of diluted goat semen in three different extenders, when the initial fructose concentration and PLA_2 activity in SP are known.

MATERIAL AND METHODS

Six crossbreed male goats two-years old were used. Extenders used were: Citrate - Egg yolk (CY): 2.37 g sodium citrate, 0.80 g glucose 2.5 % egg yolk and 100 mL distilled water (s.q.t.); TRIS- Egg yolk (TY): 3.63 g TRIS, 0.50 g fructose, 1.99 g citric acid, 2.5% egg yolk and 100 mL distilled water (s.q.t.) and industrialized coconut water[1] (bottled under the UHT system) - egg yolk (ICW-Y): 50 mL coconut water, 50 mL 2.5 % sodium citrate solution, 2.5 % egg yolk and 100 mL distilled water (s.q.t.). Twenty-four samples goat semen were collected by using an artificial (four samples/per animal). After collection, the volume of each ejaculate was measured and the sperm concentration was determined by spectrophotometry. Each ejaculate collected were divided into four aliquots, three aliquots were diluted with the three extenders (CY, TY and, ICW-Y), in the concentration of 200 x 10^6 sptz/mL. The fourth aliquot was centrifuged twice at 3.450 g/ +4 °C/20 min and the supernatant was frozen at -18 °C until the analyses for fructose concentration (colorimetric method) and PLA_2 activity (Haas *et al.*, 1968). After dilution, a sample of 300 µL from semen (fresh) in each extender was incubated at 38 °C in a water bath and assessed by the heat resistance test as regards the vigor, sperm motility and total morphological alterations (TMA) using optical microscopy. The remainder of the diluted semen was cooled at 5 ºC and, samples of each extender were used for evaluation of sperm criteria at 2, 24 and, 48 h after storage. Semen smears were made and stained with bromophenol blue and morphological alterations, were evaluated. TMA resulted from the sum of all alterations found. After the evaluation of initial fructose concentration, the animals were divided into two groups with three goats each: group I a high initial fructose concentration on SP (>710 mg/dL) and group II a low initial fructose concentration (< 480 mg/dL). Data were evaluated using the SAS v.8 (2000), according a randomized design. Sperm motility and TMA variables expressed as percentages were submitted to angular-transformed before the statistical analysis.The data was submitted for the analysis of variance (ANOVA) to evaluate the extender type effects and the concentration of seminal fructose on sperm parameters. Differences between mean values were analysed by Tukey test ($p<0.05$).

RESULTS AND DISCUSSION

The initial fructose concentration did not affect in vigor, motility and TMA sperm when CY and TY extenders were used, but when the concentration of this glicide in SP were more than 710 mg/dL there was an increase in the lifetime of sperm extended in ICW-Y. This result may be probably due the presented inappropriate or unavailable energy levels in this extender to the sperm in group II, because there is high level of carbohydrates in its composition (2.5 %), than other extenders used in this study. Furthermore, our research group has shown good results in cooling with natural coconut

[1]nutritional information: in 200 mL there are 11 g of carboidrates and 50 mg of sodium. In according to factoring, this product do not had significant quantify of proteins, fats and fiber. http://www.sococo.com.br/pt/produtos/index.asp?vArea=1&vCod=1

water (Campos *et al.*, 2004). Therefore, it is believed that the original features of natural coconut water had changed, including carbohydrate concentrations. Storage for 48 hours did not adversely affect the vigor, except in the ICW-Y from Group II that decreased within 24 h of storage **(table I)**. In extender CY, decreased motility was observed within 24 h in Group II **(table I)**. On the TMA such effect was observed in the CY of group I within 48 and 24 h in Group II **(table I)**. The ability of spermatozoa to also use glucose, fructose or mannose is due to the fact that these sugars sources enter the glycolysis cycle through the hexokinase reaction with ATP, followed by formation of lactic acid from monofosfatohexose (King *et al.*, 2006). Thus, it is believed that the depletion of sugar in animals with low fructose level is faster, and our present findings suggest that the ICW-Y extender did not sufficiently supply the energy to maintain sperm metabolic activity. In the two groups, the best motility and vigor were found in the CY and TY extenders, confirming their quality as liquid goat semen preservatives by adequately supply the energy needs of sperm (Shamsuddin *et al.*, 1999). Significant difference between the extenders was found in Group II for vigor and motility and it was more evident for vigor within 24 h and motility within 48 h **(table I)**, and the best results were observed in the CY and TY. The efficiency these extenders found in the present experiment for goat semen storage can be attributed to buffer capacity and appropriate sugar concentration, combined protective effect of egg yolk against cold shock (Cabrera *et al.*, 2005). The effect of extender on the TMA was

Table I. *Vigor, sperm motility and TMA (mean ± S.E.) in goat semen diluted in three different extenders.* (Vigor, motilidade espermática e TMA (média ± S.E.) no sêmen caprino diluído em três diferentes diluidores).

Storage Time	Group I high fructose in seminal plasma			Group II low fructose in seminal plasma		
	ICW-Y	CY	TY	ICW-Y	CY	TY
			Vigor (0-5)			
Fresh	2.96 ± 0.49^{a}	3.41 ± 0.66^{a}	3.41 ± 0.38^{a}	2.79 ± 0.44^{aB}	3.65 ± 0.41^{aA}	3.12 ± 0.75^{aAB}
2 h	2.93 ± 0.55^{a}	3.48 ± 0.83^{a}	3.15 ± 0.62^{ab}	2.75 ± 0.43^{a}	3.29 ± 0.61^{a}	3.05 ± 0.66^{a}
24 h	2.47 ± 0.37^{ab}	2.95 ± 0.97^{ab}	2.79 ± 0.66^{ab}	1.93 ± 0.84^{bB}	3.03 ± 1.21^{Aab}	2.48 ± 1.22^{ABab}
48 h	2.02 ± 0.58^{b}	2.67 ± 1.31^{b}	2.47 ± 1.09^{b}	1.51 ± 1.07^{bB}	2.47 ± 1.49^{bA}	2.23 ± 1.24^{bA}
			Sperm motility (0-100 %)			
Fresh	69.73 ± 14.77	78.48 ± 17.22^{a}	80.26 ± 10.10^{a}	66.10 ± 8.58^{a}	79.02 ± 5.85^{a}	72.92 ± 14.30^{a}
2 h	67.50 ± 14.77	73.1 ± 17.43^{ab}	74.73 ± 16.80^{a}	65.41 ± 8.37^{a}	67.08 ± 11.93^{ab}	65.28 ± 18.20^{ab}
24 h	64.16 ± 13.44	62.64 ± 25.76^{ab}	62.08 ± 21.75^{ab}	50.98 ± 23.6^{ab}	62.08 ± 24.50^{b}	58.35 ± 31.59^{ab}
48 h	58.06 ± 17.75^{1}	61.82 ± 27.57^{b}	56.24 ± 21.78^{b}	43.58 ±30.37^{2bB}	55.57 ± 33.96^{bA}	53.62 ± 30.98^{bA}
			Total morphological alteration (TMA)			
Fresh	11.66 ± 7.35^{B}	28.46 ± 17.11^{Aa}	14.67 ± 7.03^{B}	15.33 ± 6.61^{B}	25.96 ± 12.59^{Aa}	22.54 ± 13.34^{AB}
2 h	16.04 ± 14.58	21.96 ± 11.52^{ab}	17.25 ± 10.39	14.25 ± 7.53	19.54 ± 7.55	18.54 ± 10.23
24 h	14.96 ± 10.02	16.75 ± 9.42^{bc}	17.46 ± 8.76	14.75 ± 8.76	15.25 ± 9.87^{b}	16.29 ± 8.56
48 h	20.42 ± 8.78	13.25 ± 9.52^{c}	17.38 ± 8.16	14.17 ± 8.20	18.33 ± 11.82	21.96 ± 16.25

ICW-Y: industrialized coconut water-egg yolk; CY: citrate-egg yolk; TY: tris-egg yolk.
Lowercase letters: comparison between storage ($p<0.05$); Uppercase letters: comparison between extenders ($p<0.05$). Numbers: comparison between groups I and II.

observed only in fresh semen, with the largest index in the CY in both groups **(table I)**. The TMA averages were above 25 %, so that diluted goat semen in the CY extender presented abnormal parameters for use in artificial insemination. Blackshaw and Salisbury (1957) related that egg yolk stimulates the glycolytic activity of fresh semen. So, we believed that in fresh semen the addition of the egg yolk should be avoided, because it decreased seminal quality. PLA_2 activity was determined in both groups I (10.02 ± 2.43 U/mL) and II (12.09 ± 3.55 U/mL), and no significant differences were observed among the intensity of activity between the experimental groups ($p>0.05$). We found that PLA_2 activity was not related to the low quality of goat semen in Group II in this experiment.

CONCLUSION

The study concluded that when extenders are used with appropriate concentrations of carbohydrates, as the citrate and the TRIS, sperm quality remained viable regardless of the initial concentration of fructose in the goat SP. However, industrialized coconut water should not be used for storage of goat semen.

ACKNOWLEDGMENT

Thanks to FUNCAP for grants to support research and the CNPq for scholarships.

REFERENCES

Barakat, M.Z.; Abdalla, A. and Hassanein, R.R. 1972. Evaluation of fructose content of buck semen. *B Acad Pol Sci Bio*, 20: 361-363.

Blackshaw, A.W. and Salisbury, G.W. 1957. Factors influencing metabolic activity of bull spermatozoa. II. Cold-shock and its prevention. *J Dairy Sci*, 40: 1099-1106.

Cabrera, F.; Gonzalez, F.; Batista, M.; Calero, P.; Medrano, A. and Gracia, A. 2005. The effect of removal of seminal plasma, egg yolk level and season on sperm freezability of canary buck *(Capra hircus)*. *Reprod Domest Anim*, 40: 191-195.

Campos, A.C.N.; Nunes, J.F.; Monteiro, A.W.U.; Figueiredo, E.L.; Pinheiro, J.H.T.; Ferreira, M.A.L. e Araújo, A.A. 2004. Viabilidade do sêmen caprino lavado e não lavado diluído em água de coco e armazenado a 4 °C. *Rev Bras Cien Vet*, 11: 178-182.

Evans, G. y Maxwell, W.M.C. 1990. Semen y sus características. Inseminación artificial de ovejas y cabras. Editorial Acribia. Zaragoza. pp. 25.

Haas, G.H.; Postema, N.M.; Nieuwenhuizen, W. and Van Deenen, L.L.M. 1968. Purification and properties of phospholipase A from porcine pancreas. *Biochim Biophys Acta*, 159: 103-117.

King, S.S.; Speiser, S.A.; Jones, K.L.; Apgar, G.A. and Wessels, S.E. 2006. Equine spermatozoal motility and fertility associated with the incorporation of d-(+)-mannose into semen extender. *Theriogenology*, 65: 1171-1179.

Leboeuf, B.; Restall, B. and Salamon, S. 2000. Production and storage of goat semen for artificial insemination. *Anim Reprod Sci*, 62: 113-141.

Nunes, J.F. 1998. Utilização da água de coco como diluidor do sêmen de animais domésticos e do homem. *Rev Bras Reprod Anim*, 22: 109-112.

Nunes, J.F.; Corteel, J.M.; Combarnous, Y. et Baril, G. 1982. Role du plasma seminal dans la survie *in vitro* des spermatozoýds de bouc. *Reprod Domest Anim*, 22: 611-620.

Roca, J.; Martinez, E. and Vásquez, J.M. 1993. Seasonal variation in fructose and citric acid in seminal plasma of Murciana-Granadina goats. *Small Ruminant Re*s, 10: 219-226.

SAS v.8. 2000. S t a t is tical Analysis System. SAS Systems for Windows. Version 8.0. SAS Institute Inc. Cary. NC.

Shamsuddin, M.; Amir, Y. and Bhuiyan, M.M.U. 1999. Characteristics of buck semen it regard to ejaculate numbers collection intervals diluents and preservation periods. *Reprod Domest Anim*, 24: 42-46.