EFFECTS OF TWO WASHING SOLUTIONS ON SPERM SURVIVAL OF BUCKS

R. MACHADO AND A.A. SIMPLICIO

EMBRAPA-CNPC. Caixa Postal D-10, 62100, Sobral, CE. Brazil

ABSTRACT

The effects of 2 washing solutions on sperm survival throughout the freeze processing of buck semen were investigated. 514 ejaculates were collected with an artificial vagina from 39 mature bucks of Anglo-Nubian, Canninde, Brown alpine, Moxoto and Saanen, during 4 years and comprising 8 year seasons (4 rainy and 4 dry). Semen was washed if it had at least 60% of individual progressive motility (ipm) and 3.0 (in a scale 0 to 5) of vigour (v). The Krebs-Ringer-Phosphate solution or a half to half Krebs-Ringer-Phosphate solution plus 4.92% solution of sodium citrate (MOD solution) were used as washing solutions. The sperms were washed by two successive centrifugation at 2,400 pmr, during 15 min. All ejaculates that showed sediment coagulation were not frozen. The semen was thawed at 38°C during 20 sec and 5 min after thawing the individual progressive motility and vigour were evaluated. The sample that achieved ipm≥30.0% and v≥2.0 was considered useful. Efficiency of freezing (ERF) was taken between the number of useful samples divided by the total number of washed ejaculates. When MOD solution was employed no significant differences among breeds were showed ($P>0.05$), also there was no difference ($P>0.05$) between seasons, except in relation to ipm. ERF were 33.3 and 66.8% to Krebs-Ringer-Phosphate solution and MOD respectively ($P<0.05$). It can be concluded that MOD solution was more efficient than Krebs-Ringer-Phosphate solution and it allows to reduce the costs of buck semen freeze processing.

During the past 40 years several attempts have been made to achieve high kidding rates using frozen semen. However, results are within a large range of variation. Many variables are involved in freezing of semen.These which can affect its quality and fertility. Saacke (1982) discussing about the role of laboratory evaluation of semen emphasized the difficulties is experimental approach which can induce errors in fertility prediction. On other hand Seidel Jr. and Foote (1973) with a statistical approach determined the minimum number both of males and ejaculates required to promote an accurate evaluation of some semen characteristics.

Roy (1957) reported an egg-coagulation factor in buck semen. Iritani and Nishikawa (1964) concluded that the factor was an enzyme of the seminal plasma and its substrates were phospholipids. Jain and Anand (1975) found high levels of phospholipids in goat spermatozoa, allowing for instance reaction between "enzyme and substrate". This reaction promotes lysol-ecithin which has dangerous effects on sperm survival (Nunes, 1982). Removal of seminal plasma reduces the above-mentioned effects. However, spermatozoa survival and motility depends on hormonic interaction among pH, osmotic pressure, electrolitic concentration (Blackshaw and Emmens, 1951) and other substrates (Fukuhara and Nishikawa, 1972). Many

washing solutions were used to increase post-thawing survival and fertility of spermatozoa (Fukuhara and Nishikawa, 1973b; Corteel, 1975; Souza and Mies Filho, 1986; Machado *et al.*, 1989), but it was observed that shipments of semen after washing caused high wastage (Gonzales-Stagnaro, 1975; Corteel and Baril, 1975). The main purpose of this study was to evaluate sperm survival and the freezing efficiency of buck semen after washing, and further to compare the Kreb's-Ringer-Phosphate solution (Corteel, 1975) with an half-and-half solution between Kreb's-Ringer-Phosphate solution and sodium citrate isotonic solution.

MATERIALS AND METHODS

Semen was collected with an artificial vagina from 39 mature bucks, throughout a 4- year period, comprising 8 seasons (4 rainy and 4 dry). Collection rhythm varied from once to 3 times a week / male. Semen was evaluated for individual progressive motility (%) and vigour (scale 0-5 of motility degree). These were used to freeze only samples with at least 60 % of individual progressive motility and 3 of vigour.

From 514 ejaculates collected, 267 were washed by the Kreb's-Ringer-Phosphate solution (Corteel, 1975) and 247 were washed with the modified solution (Table 1).

Table 1. Washing solutions

Solution	Volume required[a] (ml)	
	KRP[b]	MOD[c]
1.15% KCl	4.0	4.0
0.90% NaCl	100.0	100.0
1.22% CaCl	3.0	3.0
2.11% KH_2PO_4	0.4	0.4
3.82% $Mg_2So_4 \times 7H_2O$	1.0	1.0
Phosphate buffer[d]	12.0	12.0
5.34% dextrose	4.5	4.5
4.92% sodium citrate	0.0	124.9

a. Add in order
b. Kreb's-Ringer-Phosphate solution
c. Modified Kreb's-Ringer-Phosphate solution
d. To prepare phosphate buffer dissolve 35.81 g NaHPO4x12H20 in 20 ml of 1.0M HCl. Make up to 1000 ml with double-distiled water.

Samples to be washed were diluted 9:1 with respective solutions and centrifuged at 2,400 rpm for 15 min. After removing the supernatant a second washing was performed. When the sperm sediment suffered adhesion to the tube, the sample was considered unsuitable and was wasted. After washing ,spermatozoa were diluted with unglycerolated skim milk at 10% concentration and large-spectrum antibiotics and glucose added. Extended semen was transferred to a cold room and cooled to +5°C in about 90 min. After this temperature was achieved an equal volume of 14% glycerolated solution was added in 3 aliquots at 10 min intervals. There was no equilibration; freezing was made through azote vaporization during 8 min (-80°C) followed by storage (-196°C). To evaluate semen 2 samples were taken from both treatments,

containing 7% glycerol and $400x10^6$ sperms/ml. The first sample was evaluated for prefreeze individual progressive motility and vigour. The second sample was observed after approximately 21 days of storage for post-thawing individual progressive motility and vigour. A shipment was considered useful when on post-thawing individual progressive motility and vigour of atleast 30.0% and 2.0, respectively, were achieved. Freezing efficiency was evaluated as frequency of washed, frozen and useful ejaculates and submitted to qui-square test. Besides, percentage values were transformed through arc-sin function and submitted to variance analysis considering the effects of breed and season of semen collection (rainy vs dry).

RESULTS AND DISCUSSION

The MOD solution had better efficiency (Table 2). It is important to pay attention to the improvement observed in the efficiency of freezing semen process of the native breed types, e.g. Caninde and Moxoto ($P<0.05$). This fact assumes a special role in the keeping of genes of a few known genotypes. General efficiency achieved with MOD solution was better than that with Kreb's-Ringer Phosphate despite breed type of males. On the other hand, the improvements observed in Anglo-Nubian (37.6% vs. 50.0%) and Brown Alpine (47.4% vs. 57.7%) were not significant ($P>0.05$). Corteel (1975) obtained an efficiency of 59.0% in Alpine and Poitevine bucks when freezing was done during breeding season in France. In the present trial the efficiency of freezing semen of Alpine breed using Kreb's-Ringer Phosphate solution is below 47.4% of Corteel's findings although the efficiency was best when compared with the other breeds now studied. In tropical climate, Gonzales-Stagnaro (1977) achieved about 60.0% of freezing efficiency. Native breed types studied did not show a satisfactory performance of freezing allowing to suppose biochemical and metabolic differences between native and exotic semen samples. On the other hand, MOD solution produced a higher number of useful ejaculates when compared to previous findings (Corteel, 1975; Gonzales-Stagnaro, 1977). The increase observed in efficiency of freezing through the use of MOD solution was supported by 2 hypotheses, the first one being the bigger input of energy substrates. Actually, the citrate as an energetic source increases oxygen uptake supporting sperm motility (Fukuhara and Nishikawa, 1973). Besides, the combination between citrate and phosphate as buffer

Table 2. Efficiency of buck semen freezing using different washing solutions

Breed (N)		Solution							
		Krebs-Ringer-Phosphate			EF1	Modified solution			EF1
		Ejaculates				Ejaculates			
		Washed	Frozen	Useful		Washed	Frozen	Useful	
Anglo-nubian	(10)	77	47	29	37.6[a]	10	06	05	50.0[a]
Caninde	(06)	34	20	07	20.6[b]	29	27	26	89.6[a]
Brown Alpine	(09)	59	42	28	47.4[a]	116	89	67	57.7[a]
Moxoto	(08)	28	21	06	21.4[b]	92	91	67	72.8[a]
Saanen	(06)	69	45	19	27.5[b]	-	-	-	-
Total	(39)	267	175	89	33.3[b]	247	213	165	66.8[a]

Values with different superscript letters deferred statistically ($P<0.05$). Efficiency of freezing (%).

washing solutions were used to increase post-thawing survival and fertility of spermatozoa (Fukuhara and Nishikawa, 1973b; Corteel, 1975; Souza and Mies Filho, 1986; Machado *et al.*, 1989), but it was observed that shipments of semen after washing caused high wastage (Gonzales-Stagnaro, 1975; Corteel and Baril, 1975). The main purpose of this study was to evaluate sperm survival and the freezing efficiency of buck semen after washing, and further to compare the Kreb's-Ringer-Phosphate solution (Corteel, 1975) with an half-and-half solution between Kreb's-Ringer-Phosphate solution and sodium citrate isotonic solution.

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medium provides a pH similar to disodium phosphate buffer (Blackshaw and Emmens, 1951). On the other hand, Fukuhara and Nishikawa (1973b) stated that the optimum pH for sperm respiration is 7.2 to 7.5 although lower pH such as 7.0 to 7.2 provides the best environment to sperm motility. Both, Kreb's-Ringer Phosphate solution and MOD solution present pH of 7.04 to 7.40 promoting a desirable hydrogen-ionic condition to spermatozoa (Fukuhara and Nishikawa, 1973).

The optimum experimental conditions stress the need to use the same semen samples divided in 2 aliquots, one to be washed by the Kreb's-Ringer phosphate solution and another washed by the MOD solution. In the present trial this procedure was not considered to be in agreement with the views of Seidel Jr. and Foote (1973) who detected a little bit of treatment differences in motility after freezing and thawing and when different ejaculates from different males were used .

The results achieved in individual progressive motility and vigour both after thawing are given in Table 3; season effects are summarized in Table 4. The interaction between season and breed was not significant (P>0.05).

Table 3. Progressive motility after thawing and vigour after thawing. Comparison between Kreb's-Ringer Phosphate and MOD solutions

Breed (N)		After-thawing			
		Progressive motility		Vigour	
		KRP	MOD	KRP	MOD
Anglo-Nubian	(10)	23.1±3.38^{a*}	25.3±11.1^{a**}	2.74±.23^{a*}	1.67±.17^{b**}
Caninde	(06)	23.5±9.19^{b*}	45.7 ±4.93^{a*}	2.21±.63^{b*}	2.85±.25^{a*}
Brown Alpine	(09)	30.9±5.64^{b*}	35.0 ±2.41^{a*}	3.13±.39^{a*}	2.23±.15^{b*}
Moxoto	(10)	10.2±5.56^{b*}	35.0 ±2.19^{a*}	1.50±.38^{b**}	2.20±.16^{a*}

KP,Kreb's-Ringer phos Phate washing solution,
MOD,Modified KRP solution.
In the same line the values with different superscript letters and in the same column values with different marks are different statistically (P<0.05).

Table 4. Effects of season on progressive motility and vigour after thawing of ejaculates washed with KRP or MOD solution.

Season	After thawing			
	Progressive motility		Vigour	
	KRP	MOD	KRP	MOD
Dry	20.2±3.00^{b**}	29.6±2.14^{a**}	2.30±.21^{a*}	1.94±.15^{a*}
Wet	25.5±1.86^{b*}	39.4±2.58^{a*}	2.63±.13^{a*}	2.29±.16^{a*}

KRP, Kreb's-Ringer Phosphate washing solution.
MOD ,Modified KRP solution.
In the same line the values with different superscript letters and in the same column values with different marks are different statistically (P<0.5).

The value achieved for individual progressive motility and vigour after post-thawing in Alpine bucks using Krebs-Ringer phosphate solution (Table 3) was comparable to that reported by Corteel and Baril (1975). Onthe other hand, a significant increase in post-thawing motility was noted by the use of MOD solution (35.0±2.41). MOD solution increased sperm survival on post-thawing, although it was unable to maintain better levels of metabolic activity of spermatozoa from Anglo-Nubian and Alpine bucks (Table 3). It can be supposed that MOD solution provides an adequate environment to spermatozoa during washing although with an expensive requirement of energy. After thawing the normal intake of energy is impaired by the extracellular increase of catabolic substances which decreases the oxygen flow with slow pH changes resulting in sperm death . After thawing individual progressive motility differences ($P<0.05$) were observed between breeds (Table 3) only in samples washed by MOD solution. Breed effects on post-thawing vigour occurred in both Kreb's-Ringer Phosphate and MOD solutions. Compared to the important increase in post-thawing parameters

of frozen semen of native breeds the response of Anglo-Nubian was poor (Table 3). However there was an increase ($P>0.05$) in freezing efficiency (Table 2). Corteel and Baril (1975) described seasonal changes in frozen semen of Alpine and Poitevine reared under temperate conditions. The present study was made in tropical conditions where there are not photoperiodic changes strong enough to depress reproductive efficiency of male goat (Nunes, 1982). However, difference ($P<0.05$) was observed among rainy and dry periods (Table 4) possibly due to the adequate schedule of additional feeding supplied throughout the year associated to the decrease of rain stress to browsing behaviour. It can be concluded that Kreb's-Ringer Phosphate solution is not good for washing semen of Brazilian native breed of goats. On the other hand freezing efficiency and post-thawing sperm survival achieved with MOD solution presented significant increases. MOD solution was ineffective to obtain good results on freezing semen of Anglo-Nubian bucks.

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