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EFFECT OF FILTRATION ON BOER GOAT SEMEN QUALITY DURING CRYOPRESERVATION

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SUMMARY

Sephadex G-15, cotton and glass wool filters were designed to remove abnormal and nonmotile goat spermatozoa from extended ejaculate before cryopreservation. Filtration improved semen quality in filtered samples than in the unfiltered ones both prior to and after freezing by removing most of dead spermatozoa. Addition of 50% killed spermatozoa to semen sample reduced sperm motility after freezing and thawing. Filtration of the deteriorated sample increased percentage of motile sperm during cryopreservation. Sephadex G-15 showed superior results than glass wool and cotton filters in terms of sperm motility and live spermatozoa with intact acrosomes before and after freezing.

Keywords: Goat semen, filtration, cryopreservation

INTRODUCTION

In some instances poor quality semen is obtained under field conditions. Insemination with a double dose or more of low quality semen seems inappropriate because dead spermatozoa have a negative toxic effect on the remaining normal sperm population (Lindemann *et al.* 1982). Therefore, removal of dead and abnormal sperm from low quality ejaculate is a more logical approach. The first pioner work for separation of motile from nonmotile spermatozoa was performed by passing diluted semen through a layer of small glass beads by Bangham and Hancock (1955). Other methods of sperm separation have been developed using pyrex beads (McGrath *et al.*,1977), bovine serum albumin gradients (Dixon *et al.*, 1980 and Goodeaux and Kreider, 1978), glass wool (Maki-Laurila and Graham, 1968), Newtonian gels (Luderer *et al.*, 1982), Sephadex gels (Graham *et al.*, 1976; Graham and Graham, 1990), and a swimup method (Parrish *et al.*, 1986).

In the present study, Boer goat semen was filtered through Sephadex gel, cotton and glass wool filters before freezing. The efficiency of these fitters was evaluated by filtering a deteriorated semen sample containing 50% killed spermatozoa in terms of sperm motility, concentration, percentages of dead and live sperm and presence of acrosmes during cryopreservation.

MATERIALS AND METHODS

Goat semen was obtained from two normal bucks by using an artificial vagina. Semen ejaculates with motility scores over 70% were pooled before use in the experiments. Semen (2 ml) was divided into two equal parts. One part was used as normal (N). The other half was replaced by 50% (v/v) killed semen sample. Killing of spermatozoa was performed by placing a semen sample in hot water bath (70-80°C) for one minute for 2-3 times. The semen sample containing 50% dead spermatozoa was considered as deteriorated (D). Motility of the two semen samples (N and D) was determined under light microscope equipped with warm stage (37°C). Both semen samples were extended (1:12) gradually in Tris-citrate-yolk buffer before freezing.

The extender was composed of 3.786 gm Tris (hydroxy methyl amino methanol), 2.115 gm citric acid, 20 ml egg yolk, 6.8 ml glycerol and antibiotics (1000 I.U./ml of penicillin and 5 mg /ml of streptomycin) per 100 ml.

Three equal parts (2 ml) from each extended semen samples (N and D) were passed through three designed filters: Sephadex, glass wool and cotton. The recovered filtrate from each filter and from N and D samples as well were packed in 0.25 ml straws and equilibrated at 4°C for at least 4 hrs before freezing in LN2.

Preparation of filters

Sephadex filter: dry Sephadex G-15 (0.2 gm) were distributed over a thin layer of glass wool placed in the bottom of a pasteur pipette to prevent Sephadex loss and 1 ml of Tris-citrate buffer was added. The Sephadex particles were allowed to swell 0.5 to 1 hrs at room temperature (24°C).

Glass wool and cotton filters: suitable amount of glass wool or cotton were inserted into pasteur pipette to the hight of 1 ml mark on the pipette without hard packing in order to allow suitable release of the filterats. A small piece of tygon tubing was attached to the tip of each piptte and clamped shut. Each tubing was inserted into a filtrate collecting graduated tube .

Data collection

Sperm counting was carried out by using hemocytometer (Thoma neu) slide on samples before and after filtration.

Sperm staining for determination of percentage live and dead, with or without intact acrosomes by the method of Didion *et al.* (1989) was performed on samples before and after filtration and after freezing and thawing. Each experiment was repeated five times.

Statistical Analysis

Data were analyzed by one way analysis of variance and Duncan's multiple-range test according to Snedecor and Cochran (1980). To reveal the interation effect on the experiment, data were analyzed by two-way analysis of variance (SAS, 1985) according to the following model:

$$Y_{ijk} = \mu + P_i + T_j + P*T_{ij} + e_{ijk}$$

where:

Y_{ijk} = The observation, μ = The overall mean, P_i = Effect of type of semen (N or D), T_j = Effect of treatment (filtration or not).

$P*T_{ij}$ = Interaction of type and treatment, e_{ijk} = Random error

RESULTS

Analysis of variance (Table 1) shows significnat ($P < 0.01$) effects for type of semen and treatment (filtration) on all studied parameters. However, the interaction effect (P × T) was only significant with prefreeze and postthaw sperm motility ($P<0.05$) and with prefreeze total dead sperm ($P < 0.01$).

Table 1. Analysis of variance and mean squares for sperm motility prefreeze (pre-F.M), postthaw (post-T.M), prefreeze total live sperm (pre- L.S), total dead sperm (D.S) and postthaw total live sperm, total dead sperm.

				Prefreeze		Postthaw	
SOV	df	pre-F.M	post-T.M	L.S	D.S	L.S	D.S
Type (P)	1	6002.5**	4730.6**	2387.0**	2402.5**	4558.2**	4558.2**
Treat. (T)	3	495.0**	1595.6**	977.2**	986.1**	799.1**	799.1**
P * T	3	100.8	117.3*	106.6	108.3**	35.9	35.9
Error	32	23.4	27.5	51.8	16.3	6.8	41.1

P* < 0.05, P ** < 0.01

The percentage of motile sperm and sperm concentration of the unfiltered and filtered extended goat semen during cryopreservation are presented in Table 2. Normal semen samples showed higher ($P< 0.05$) motility score than the deteriorated samples containing 50% killed sperm, both prior to and after freezing. Semen samples passed through the three filters contained more motile cells than the unfiltered samples. This trend was only significant ($P< 0.05$) using Sephadex filter in the normal semen samples however, was significant ($P<0.05$) in the deteriorated semen samples in all filters used (Table 1 & 2). This means that filtration by using any type of the designed filters was effective to remove dead cells in the case of the deteriorated sample. Motility of spermatozoa varied significantly ($P<0.05$) between semen samples postthawing. This indicated that the quality of the frozen samples varied prefreezing since all samples were exposed to the same handling condition and freezing process. Sephadex filter allowed more motile sperm with intact acrosomes to pass through (Table 2). Semen filtration through Sephadex filter showed higher motility values than cotton and glass wool filters. Sephadex filter significantly ($P<0.05$) improved samples motility than cotton and glass wool filters in the normal sample before freezing.

Freezing process caused a pronounced reduction in sperm motility after thawing in all samples. However, filtration of semen samples (N or D) before freezing reduced the decrease in sperm motility postthawing. This was due to removal of most dead cells from filtered samples before freezing which in turn yielded better sperm motility scors postthawing. Filtration caused a significant ($P < 0.05$) reduction in sperm concentration in the filtrate. This was due to preventing of dead sperm from passing through the designed filters (barriers). In case of the deteriorated sample, more sperm were trapped in the filters which caused more reduction in sperm count in the filtrates than the case in the normal sample (Table 1). Table 3 shows the features of spermatozoa in filtered and unfiltered extended goat semen during cryopreservation. The deteriorated semen sample contained significantly more ($P<0.05$) dead cells with

and without intact acrosomes than in the normal sample both prefreezing and postthawing. Filtration through all filters reduced ($P<0.05$) these values than in the deteriorated sample (control). Generally, filtration caused an increase in percentages of live spermatozoa with intact acrosomes in both normal and abnormal samples during cryopreservation. Extended semen passed throug Sephadex filter showed higher ($P<0.05$) percentages of live spermatozoa with intact acrosomes than that in cotton and glass wool filters both in normal and deteriorated samples during cryopreservation. Sephadex filter to a high extend trapped most of dead and abnormal spermatozoa from filtered samples which in turn improved sample performance during freezing than the case in cotton and glass wool filters. This was evidenced by the reduction in sperm concentration in Sephadex filtrate than that in the other two filters (Table 1).

DISCUSSION

Semen filtration improved sperm motility before freezing by removing most of dead cells from samples. The filtered samples contained approximately 80% motile sperm in the normal sample and 65% in the deteriorated sample. Similar results have been reported when Sephadex G-15 filter was used to separate bull spermatozoa (Graham and Graham, 1990). Maki-Laurila and Graham (1968) reported 85 to 95% motile sperm after glass wool filtration for bull semen samples originally contained 30 to 40% motile sperm.

Table 2. Effect of filtration on motility (%) and concentration ($x10^7$/ml) of prefreeze and posttow extended goat spermatozoa.

Treatment of semen	Stage of Cryopreservation			
	Prefreeze			Post-thaw Motility
	Motility	Concentration		
		infiltrate	% from prefiltered	
Unfiltered				
N	82±2.6^{a}	41.3±1.9^{a}	---	49±2.9^{abc}
D	48±3.7^{d}	36.5±2.6^{ab}	---	17±3.0
Filtered				
SN	92±1.2^{c}	29.5±0.9^{c}	71.4	72±2.0
CN	85±2.2^{a}	30.0±3.6^{bcd}	72.6	54±2.5^{bc}
GN	88±1.2^{a}	34.5±4.9^{abce}	83.5	63±1.2^{c}
SD	71±2.5^{b}	17.5±1.9^{f}	47.9	53±2.0
CD	63±1.2^{e}	24.1±1.9^{def}	66.0	36±2.5
GD	67±1.2^{b}	19.3±1.8^{f}	52.9	45±2.2^{a}

^{a,b,c,d,f} Means±S.E on the same column with different letters differ ($P < 0.05$). N = Normal sample, D = Deteriorated sample, S = Sephadex, C = Cotton, G = Glass wool

Reduction of sperm number in the filtrate than in the prefiltered sample may be due to trapping of dead sperm which may clog the filter. Graham and Graham (1990) found that filtration of bull ejaculates with a high percentage of abnormal sperm, induced by scrotal insulation or deteriorated by freeze killing, resulted in a significant increase in the percentage of motile sperm with normal morphology and a reduction

in sperm concentration in the filtrate. They reported an increase in percentages of sperm with normal head shape and normal acrosomes after filtration. These results are in agreement with our findings.

The efficiency of such filter depends on percentage of motile sperm with normal morphology in the filtrate. Sephadex G-15 filter was superior to glass wool and cotton filters in this regard.

Table 3. Effect of filtration on spermatozoa features in prefreeze and postthaw extended goat semen.

Treatment of semen	Prefreeze					
	Live		Total	Dead		Total
	Ac_i	Ac_l		Ac_i	Ac_l	
Unfiltered						
N	66.6±4.9abc	7.6±1.0a	74.2	15.0±2.4a	10.8±1.4a	25.8
D	41.2±3.2d	12.4±0.5c	53.6	22.0±1.9b	24.4±2.5c	46.4
Filtered						
SN	90.4±2.9	0.8±0.4	91.2	7.6±0.5c	1.2±0.4	8.8
CN	79.2±4.3ac	8.8±1.4bc	88.0	7.4±1.0c	4.6±0.8ab	12.0
GN	82.8±2.5b	3.6±0.9a	86.4	10.8±1.4c	2.8±0.7b	13.6
SD	80.0±2.9b	4.2±0.7a	84.2	12.2±1.1ac	3.6±0.9ab	15.8
CD	51.8±1.9de	15.6±1.9c	67.4	10.8±2.0c	21.8±1.2c	32.6
GD	66.2±3.1ce	6.6±0.7ab	72.8	14.0±1.8a	13.2±1.3a	27.2
Postthaw Unfiltered						
N	40.2±1.7a	11.2±1.2a	51.4	20.4±1.5a	28.2±2.2a	48.6
D	12.4±1.4	12.2±1.5a	24.6	47.8±2.2	27.6±1.9a	75.4
Filtered						
SN	64.2±3.2	4.6±0.7b	68.8	16.6±1.9	15.2±1.1b	31.2
CN	43.4±2.2a	7.6±1.0bc	51.0	25.2±3.0ab	23.8±1.7ac	49.0
GN	52.0±1.7	5.2±0.8b	57.2	24.0±2.3a	18.8±0.9e	42.8
SD	44.0±2.4a	3.8±0.8b	47.8	38.8±1.8c	13.4±1.5b	52.2
CD	20.2±1.3b	11.6±1.2a	31.8	32.4±2.8bc	35.8±1.9	68.2
GD	29.8±1.5b	9.0±1.7c	38.8	39.8±1.9c	21.4±1.8ce	61.2

a,b,c,d,e Means±S.E. on the same column in each stage with differnt letters differ ($P < 0.05$)
Ac_i = Intact acrosome, Ac_l = Lefted acrosome, A = Normal sample, D = Deteriorated sample, S = Sephadex , C = Cotton, G = Glass wool.

The exact mechanism of removal of dead and abnormal spermatozoa by Sephadex G-15 filter is not understood. Graham *et al.* (1976) suggested that filtration of spermatozoa on a Sephadex column appears to be physico-chemical reaction with Sephadex particles providing a barrier, allowing immotile or dead sperm cells to agglomerate. Furthermore, they found that inactive boar spermatozoa did not pass through the Sephadex filter, even after washing with 500 times of their volume. However, when a stimulant such as caffeine or cyclic AMP is added to it, the motile cells passed through the filter immediately. This indicated that passing of spermatozoa through the Sephadex filter depend on their active motility. Moreover

Lodhi and Crabo (1984) suggested that damage of sperm membrane may lead to the exposure of different macromolecules which might bind to the Sephadex particles. In addition, it is speculated that after the death of spermatozoa, positively charged components appear on the sperm membance, as reported following the acrosome reaction (Farooqui, 1983) which interact with negatively charged gel particles and are trapped. These speculations may explain our findings since fewer number of live spermatozoa without acrosomes were found in the Sephadex and glass wool filtrates than in the cotton filtrate.Sperm separation through cotton (cellulose) filter seems to be depending on sperm motility since high number of spermatozoa without acrosomes was found in the filtrate.

These result is in turn reflected on the high sperm motility scores observed in frozen samples after thawing. The lower sperm motility score observed in glass wool filtered samples after freezing and thawing than in samples filtered through Sephadex could be due to glass wool residues which appeared in the effluent (Daya and Gwatkin, 1987). Sherman *et al.* (1981) reported that glass wool column induced ultrastructural damage to the membrane and the acrosome of filtered spermatozoa resulting in loss of their motility.

The probability of fertilization is governed by the total concentration, the progressive motility, the morphology and the half-life of spermatozoa (Van Duijn, 1965). Insemination with a double dose or more of low quality semen seems inappropriate because dead spermatozoa have a negative toxic effect on the remaning normal sperm population (Lindemann *et al.*, 1982). Therefore, removal of dead and abnormal sperm from low quality ejaculate is a more logical approach for maximizing the genetic potential of a sire.

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تأثير عملية الترشيح على صفات السائل المنوى فى ماعز البوير خلال التجميد

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صممت ثلاثة فلاتر (سفادكس-١٥، قطن، صوف زجاجى) لازالة الحيوانات المنوية الشاذة والغير متحركة فى السائل المنوى المخفف للماعز قبل التجميد. حسنت عملية الترشيح صفات السائل المنوى فى العينات المرشحة عنها فى الغير مرشحة قبل أو بعد التجميد.

إضافة نسبة ٥٠٪ حيوانات منوية ميته إلى العينة خفضت حيوية العينة بعد التجميد والإسالة. ترشيح هذه العينة السيئة أدى إلى زيادة نسبة الحيوانات المتحركة خلال عملية التجميد.

أظهر فلتر السفادكس-١٥ نتائج متفوقة عن كلا من الصوف الزجاجى والقطن بالنسبة لحيوية الحيوانات المنوية وسلامة الأكروسوم قبل وبعد التجميد.