

Effect Of Glass Wool Filtration Method On Frozen-Thawed Dog Semen

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Summary: The aim of this study was to evaluate the effects of the glass wool filtration on motility and morphology of post-thaw quality of dog semen. Two German Shepherd Dogs were used as material. Ejaculates were diluted with Tris-citric acid-egg yolk and Sodium citrate-egg yolk extenders at 1:1 ratio including a final 4% glycerol concentration. Semen were frozen in 0.5 ml straws and thawed in a water bath at 37°C for 30 sec. Pasteur pipettes were used as columns. Each column was constructed with 20, 40 and 80 mg glass wool and at 2, 3 and 4 cm heights, respectively. Filtrates were examined before and after filtration for motility, acrosomal, other and total defects. When the filtration methods were compared, the highest improvement percentage in motility was found in column filled with 40 mg glass wool. On the other hand extenders did not have any effect on motility and other morphological defects. But significantly interactions were found between spermatological parameters and filtration techniques. In conclusion, glass wool filtration techniques could be used successfully for filtration of frozen-thawed dog semen.

Key Words: Frozen dog semen- glass wool –filtration.

Dondurulmuş-Çözündürülmüş Köpek Spermaları Üzerine Glass Wool Filtrasyon Yönteminin Etkisi

Özet: Bu çalışmada dondurulmuş-çözündürülmüş köpek spermalarını motilite ve morfolojisi üzerine glass wool filtrasyon yönteminin etkilerinin araştırılması amaçlanmıştır. Materyal olarak 2 adet Alman çoban köpeği kullanıldı. Ejakulatlar Tris-sitrik asit- yumurta sarısı ve Sodyum sitrat-yumurta sarısı sulandırıcıları ile finalde %4 gliserol içerecek şekilde 1.1 oranında sulandırıldılar. Sperma 0.5 ml.lik payetlerde donduruldu ve 37°C'lik su banyosunda 30 sn'de çözündürüldüler. Kolon olarak pasteur pipetleri kullanıldı. Her bir kolon 20 mg, 40 mg ve 80 mg ağırlığında ve sırasıyla 2, 3 ve 4 cm.lik genişlikten oluşturuldu. Filtratlar filtrasyon öncesi ve sonrasında motilite, akrozomal, diğer ve toplam defektler yönünden muayene edildiler. Filtrasyon yöntemleri karşılaştırıldığında, en iyi motilite ve ilerleme yüzdesi 40 mg.lık glass wool'dan oluşan kolonda bulundu. Diğer yandan sulandırıcıların motilite ve diğer morfolojik defektler üzerine herhangi bir etkisinin olmadığı saptandı. Fakat filtrasyon teknikleri ile spermatolojik özellikler arasında önemli ilişki bulundu. Sonuç olarak, donmuş-çözündürülmüş köpek spermalarında glass wool filtrasyon tekniğinin başarılı bir şekilde kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Donmuş köpek spermaları, glass wool, filtrasyon.

Introduction

Good quality spermatozoa are always desired for cryopreservation, artificial insemination and in-vitro embryo production. Many techniques such as percoll density gradients, swim up

method, glass wool filtration and glass bead filtration have been used for separating non-motile and dead spermatozoa from human^{5,8,12,13,20,25} and bovine semen^{2,3,7,10,31,32}. The main purposes of several sperm selection methods have been reported to obtain highly motile spermatozoa and

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increase the yield and characteristics of spermatozoa. Dead and abnormal spermatozoa reduce the fertility rate on artificial insemination procedures and they have adverse effect on companion cells^{18,30}. Pereira et al²⁴ reported that glass wool filtration is a useful means of eliminating a proportion of the immotile spermatozoa. Because motility is an essential criteria to achieve good fertilisation.

Dog semen should not possess more than 20 to 30 % abnormal spermatozoa to achieve optimum fertility¹⁴. The fertility of dog semen before and after freezing thawing is based on parameters of semen quality such as motility, percentage of normal acrosomes and morphology²².

Previous studies were undertaken as to compare the efficiency of sperm selection methods on frozen-thawed bovine semen but practically there was no data demonstrating the effect of glass wool filtration on frozen-thawed dog semen motility and morphology

Chandranan et al⁴ reported an increase from 77.5% to 87.2% in good quality and 61.3% to 76.9% in poor quality crossbred bull semen after filtration through a glass wool column of a height 2 cm. The increase in motility from 43% to 62% in frozen-thawed bovine semen was lower than reported by Maki-Laurila and Graham¹⁹ who obtained 85% to 95% motility after filtration of fresh bovine semen which had rates of the motility was 30% to 40% before filtration. But no data demonstrated the effect of glass wool filtration on frozen-thawed dog semen motility and morphology.

The objectives of this study were the effects of the glass wool filtration on motility and morphology of frozen-thawed dog spermatozoa and efficiency of the filtration columns in improving semen quality.

Materials and Methods

Animals:

Two German Shepherd dogs were used in this study. Their fertility and health were known. The dogs had been sexually rested for at least a week before the study.

Semen processing, freezing and filtration:

Good quality ejaculates (at least 80 % motility, less than 20% morphological abnormalities) were collected from two German Shepherd dogs

by digital manipulation in the presence of teaser bitch. Three ejaculates were obtained from each dog during 2 weeks. Semen samples were diluted with Tris-citric acid-egg yolk (T-EY; Tris hydroxymethyl-aminomethane 36g/l, citric acid 19 g/l, fructose 0.5 g/l, egg yolk %20 (V/V)) and Sodium-citrate-egg yolk (SC-EY; sodium citrate 14.5g/l, glucose 12.5 g/l, glycine 9.3g/l, %20 (V/V)) extenders 1:1 ratio including final concentration 4% glycerol. T-EY and SC-EY extenders contained 20% (v/v) egg yolk. Diluted semen samples were frozen in 0.5 ml straws. Straws frozen semen from three ejaculates were pooled in two different canisters according to extenders. Straws were thawed randomly from two canisters according to extenders and filtered for three filtration groups (20, 40 and 80 mg). Total 28 straws (9,9 and 10 straws; 20 mg, 40 mg and 80 mg, respectively) were thawed in water bath at 37°C for 30 sec in this study. To separate the motile spermatozoa, frozen-thawed dog semen was filtered through the glass wool (Glass Wool Fiber, Sigma) columns. Pasteur pipettes were used as columns. Three types of filtration columns were used. Each column was constructed with 20, 40 and 80 mg glass wool weight and at 2, 3 and 4 cm heights, respectively. Then columns were rinsed one times with 0.25 ml 0.9% NaCl solution to remove loose glass wool fibres and other particles. After filtration, filtered semen samples were collected in tubes at 37°C. Before and after filtration semen samples were examined for motility, acrosomal, other (head, mid-piece and tail) and total morphological defects. The percentage of progressively motile spermatozoa was estimated by microscopic examination at X400 magnification on pre-warmed slide. The percentages of acrosomal, other and total morphological defects were evaluated after staining with Giemsa. Thereafter, a total of 200 spermatozoa per slide were examined under light microscope (X1000) to study the sperm acrosome, other and total morphological defects.

Improvement percentage determination

The percentage of improvement in motility, acrosomal, other and total morphological defects as a result of treatment with sperm selection methods was calculated according to:

$$\frac{\text{Semen parameter after treatment} - \text{semen parameter before treatment}}{\text{Semen parameter before treatment}} \times 100$$

Statistical analysis:

Statistical analysis of data was performed by ANOVA (SPSS, 10.0). Significance between treatment methods was determined by the “Least Significant Difference” method. All data are presented as mean \pm SD.

Results

Motility, other and total morphological defects before and after filtration are given in Table I. Effects of extender, weights of column and their interaction on the semen parameters were shown in Table II. There were no significant differences in motility rates of frozen-thawed semen before filtration. On the other hand, the highest motility (69.12 \pm 2.5%) was found with 40 mg glass wool column ($p<0.05$) when 20mg, 40mg and 80 mg glass wool columns were compared after filtration.

Acrosomal and total morphological defects were significantly decreased after filtration. But morphological defects were not change according to filtration techniques (Table I). On the other hand there were statistically significant differences in other morphological defects ($P<0.05$) in before filtration between 40 mg and 80 mg of glass wool columns.

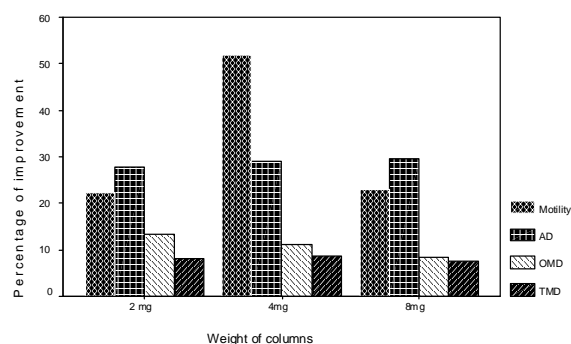
Table I. Motility, Acrosomal Defect, Other and Total Defects Before and After Filtration and Improvement Percentage in Function of Different Columns. Data Expressed as mean \pm SD

Semen parameter	n (thawed straw)	Columns	Before filtration	After filtration
Motility (%)	9	20 mg	51.37 \pm 2.5 ^{Ab}	61.94 \pm 2.5 ^{Ba}
	9	40 mg	46.11 \pm 2.5 ^{Ab}	69.12 \pm 2.5 ^{Aa}
	10	80 mg	49.50 \pm 2.4 ^{Ab}	60.50 \pm 2.4 ^{Ba}
Acrosomal defect (%)	9	20 mg	18.20 \pm 1.3 ^{Aa}	12.35 \pm 1.3 ^{Ab}
	9	40 mg	19.20 \pm 1.3 ^{Aa}	13.0 \pm 1.3 ^{Ab}
	10	80 mg	20.04 \pm 1.2 ^{Aa}	13.88 \pm 1.2 ^{Ab}
Other morphological defect (%)	9	20 mg	9.90 \pm 1.1 ^{ABa}	8.0 \pm 1.1 ^{Aa}
	9	40 mg	13.10 \pm 1.1 ^{Aa}	10.3 \pm 1.1 ^{Aa}
	10	80 mg	9.80 \pm 1.1 ^{Ba}	8.1 \pm 1.1 ^{Aa}
Total morphological defect (%)	9	20 mg	28.13 \pm 1.7 ^{Aa}	20.36 \pm 1.7 ^{Ab}
	9	40 mg	32.30 \pm 1.7 ^{Aa}	23.3 \pm 1.7 ^{Ab}
	10	80 mg	29.83 \pm 1.6 ^{Aa}	21.98 \pm 1.6 ^{Ab}

^{a,b} Different superscript letters in the same row indicate significant differences $P<0.05$

^{A,B} Different superscript letters in the same column within same characters indicate significant differences $P<0.05$

The highest improvement percentage in motility was found in column filled with 40 mg glass wool (Figure 1). There were no significant differences in improvement percentage of acrosomal, other and total morphological defects in all glass wool columns (Figure 1).



AD=Acrosomal defect, OMD=Other morphological defect, TMD=Total morphological defect

Figure 1:
Improvement Percentage of All Semen Parameters According to Weights of Column

There were no significant interactions between extenders and filtration techniques in all spermatological parameters. But significant interactions were found between spermatological parameters and filtration techniques (Table II).

Table II. Effect of Extender, Weights of Column and Their Interaction on the Semen Parameters (ANOVA). Data Expressed as mean \pm SD

	Motility	AD	OMD	TMD
Extenders	NS	*	NS	*
Weights of column	**	**	*	**
Extenders x weight of column	NS	NS	NS	NS

* $P<0.05$ ** $P<0.001$
AD=Acrosomal defect, OMD=Other morphological defect, TMD=Total morphological defect

Discussion

In this study, it was found that glass wool filtration method might have successfully used on frozen-thawed dog semen. Percentage of motility and morphological defects (acrosomal and total) were significantly decreased after filtration. This result indicated that glass wool filtration technique was increased to potential fertility of se-

men. Linde-Forsberg^{16,17} reported that there is a strong relationship between fertility post thaw motility and the percentage of normal acrosomes.

From the results of this study, we conclude that glass wool filtration method might be useful for the separation of motile spermatozoa from frozen-thawed dog semen. Potential fertility is low in dog semen when a high percentage of spermatozoa have primary and secondary abnormalities^{6,14,23}. In this study, glass wool filtration proved to be quite effective in morphologically abnormal spermatozoa. (acrosomal and total morphological defects). Glass wool columns effectively reduced acrosomal and total defects. Similar reduction of this abnormalities after glass wool filtration has been previously reported in bull^{1,4,24,31,33-35}, stallion^{28,29} and mouse¹⁵.

Our results indicated that glass wool filtration improved the after filtration motility as well as the percentage of spermatozoa with normal acrosomes and reduced abnormal spermatozoa compared with before filtration. Acrosome integrity is one of the main parameters in evaluation of fertility in dogs²². Acrosomes include hydrolytic enzymes necessary for oocyte penetration^{26,27}. The release of toxic substances by dead and abnormal spermatozoa is believed to adversely affect the fertilisation potential of their companion cells^{1,11}. Therefore, elimination of undesirable spermatozoa from frozen-thawed dog semen with glass wool filtration technique can be used for semen quality

However, we could not demonstrate a statistically significant improvement in the percentage of acrosomal, other and total defects among different columns after filtration. Effect of column filtration upon the semen quality parameters of fresh dog semen has been studied²¹. No study has documented the effect of glass wool filtration on morphological characterisation of frozen-thawed dog semen. Therefore, we assumed that filtered semen might have better fertilisation potential due to higher motility, normal acrosomes and lower sperm abnormalities than nonfiltered semen.

We found the highest improvement percentage of motility was in 40 mg column. In addition, there were no significant differences in acrosomal and total defects among all columns in before and after filtration.

In this study, percentage of acrosomal, other and total morphological defects were not

affected from the height and diameter of the column. These findings were in agreement with results reported by Cisale et al³. Results of present study demonstrated that post-thaw quality of dog semen was greatly improved after filtration. In our study indicated that the glass wool method was simple but effective.

Anzar & Graham³ and Fernandez et al⁹ have proposed that retention of spermatozoa in filtration columns depends fundamentally upon individual sperm motility and on the characteristics of the fluid in which the spermatozoa swim.

In conclusion, after filtration, the filtered spermatozoa have significantly better progressive motility. The glass wool method is promising technique for selecting motile cryopreserved dog sperm and this method resulted in greater sperm percentage motility and greater percentage normal morphology. Such a method may have clinical use for semen preparation to intrauterine insemination and in-vitro fertilisation and may be an alternative for improving the low quality of fresh and frozen-thawed dog semen.

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