Rabbit Semen Characteristics after Passing through Designed Filters

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Abstract: This study was conducted to improve the quality of rabbit semen by removing dead, immotile and abnormal spermatozoa using five different filtration methods and swim-up technique. Six different semen filtration methods (Sephadex-G15, Albumin, Cotton, Synthetic Fiber, and Sand) and swim-up technique were used. Ten matured rabbit bucks were used for semen collection. Raw and filtered semen samples were evaluated for motility, concentration, and curvilinear velocity by computer assisted sperm analysis (CASA) and membrane integrity by acrosome staining method. Analysis of variance showed significant differences (P≤0.05) due to the combination between filter methods and sperm fractions in progressive motility. The Filtration process improved (P≤0.05) sperm progressive motility than before filtration. Higher sperm motility scores were found in semen fractions two and three (P≤0.05) than that in semen fraction one and in the control sample. High positive correlations were found between the studied semen quality parameters. It could be concluded that most semen quality parameters were improved significantly in all used filters. Sephadex-G15, Sand and Swim-up selection techniques could be more efficient to be practiced routinely in rabbit semen handling. Also, both second and third filtered fractions could be effectively used in artificial insemination (AI) programs.

Keywords: Rabbit; Semen quality; Filtration; Designed filters

INTRODUCTION

Evaluation of semen could provide a precise indication of the fertilizing ability of spermatozoa. The most relevant parameters correlated with the fertility rate are the number of deposited spermatozoa and their motility, although the use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen (Love and Kenney, 1998).

Insemination with poor quality semen or even a double dose or more of low-quality semen seems inappropriate because dead spermatozoa have detrimental and toxic effects on the remaining normal sperm population (Lindemann *et al.,* 1982). Rabbit's seminal plasma contains different types of particles, which affect the spermatozoa behavior during its journey along the female reproductive tract. The first pioneer work for separating immotile spermatozoa through a layer of tiny glass beads (Bangham and Hancock, 1955). Further methods were used such as Bovine serum albumin gradients (Goodeaux and Kreider, 1978), Glass wool (Ayoub *et al.,* 1996), Newtonian gels (Luderer *et al.,* 1982), Sephadex gels (Graham *et al.,* 1976; Graham and Graham, 1990; Ayoub *et al.,* 1996) and Swim up method (Parrish *et al.,* 1986).

The objective of the present work was conducted to study the effect of different filtration methods on the post-filtration quality of rabbit semen by removing dead, immotile and morphologically abnormal spermatozoa.

MATERIALS AND METHODS

This experiment was carried out at the Animal Production Department Laboratory and Rabbitry of the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Ten Chinchilla mature bucks were used in semen collection. Animals were healthy and free of any internal parasites or skin diseases. Age of bucks ranged from 8-10 months. After semen collection by artificial vagina, measurements were taken immediately such as ejaculate color, volume, pH, total motility, progressive motility, dead/live count, and sperm cells concentration per ml. The semen extender used in extending rabbit semen was Tris buffer prepared by dissolving 3.605 g Tris, 2.024 g citric acid and 1.490 g fructose in 100 ml distilled water.

Procedures of semen filtration: -

***Sephadex G-15*:** A Sephadex suspension was prepared by hydrating Sephadex G-15 (Sigma-Aldrich ® GE17-0020-01) for at least 24 h in sodium citrate 3% (v/v). The filtration column was prepared according to (Januskauskas *et al.,* 2005) in a 10 ml disposable plastic syringe and plastic tubing was attached to the tip of the syringe and clamped. A small amount of cotton (0.0664 g) was compressed with the plunger to the bottom of the syringe to prevent loss of Sephadex particles. Sephadex was gently layered over the cotton and allowed to settle for 3 min. The extended semen was gently layered on the column and filtered through the column at room temperature (25–28 °C).

***Albumin gradient*:** Three concentrations of bovine serum albumin (BSA) were prepared in tris buffer (4, 6 and 10%, respectively). 2 ml from each were loaded in 10 ml syringe connected to a polyethylene tube shut with a clamp and incubated at room temperature. A 0.5 ml of semen was placed at the top of the BSA for 60 min, three fractions were collected (2 ml/fraction) and examined for semen evaluation parameters.

***Sand*:** One gram of sand was sieved using 10 mm sieve and washed 3 times with distilled water and 3 times with saline, then sterilized for 30 min at 100 °C. A small amount of cotton (0.0664 gm) was compressed with the plunger to the bottom of the syringe to keep sand inside the syringe. 3 ml of extended semen was put at the top of the sand column while closing the roller clamp for 15 mins and then three fractions were collected (1ml/fraction) and examined.

***Synthetic Fibers*:** 0.08 gm of soft synthetic fiber were put at the bottom of a plastic syringe and 3 ml of

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extended semen was put at the top while closing the roller clamp of the IV tubing for 15 mins and then three fractions were collected (1ml/fraction) and examined.

***Cotton*:** 0.1 g of fluffy cotton were put at the bottom of a plastic syringe (without compressing) and 3 ml of tris buffer were put at the top while opening the roller clamp (Ayoub *et al.,*1996) the aim of this step is to wet the cotton to prevent cotton-semen absorption. 3 ml of extended semen was layered at the top of cotton, while closing the roller clamp for 15 mins and then three fractions were collected (1ml/fraction) and examined.

***Swim up technique*:** Eight ml of tris buffer placed in a 15 ml test tube in a 37°C water bath and 0.5 ml of semen were injected carefully at the bottom of the test tube and incubated for 1h. Three fractions (0.5 ml each) were taken carefully, from the top of the test tube, at 15, 30 and 60 minutes after incubation, respectively. All collected fractions were evaluated.

Semen evaluation: Extended semen samples (before filtration) and all filtered fractions were evaluated subjectively under high power (400X) microscopy and through CASA determination for sperm concentration, and other sperm characteristic patterns.

Statistical analysis: Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 2003). Differences among means were detected using Duncan's new multiple test (Duncan, 1955).

RESULTS

Results presented in Figure (1) showed the percentage of progressive motility of rabbit's spermatozoa before and after filtration. The analysis of variance showed significant differences (P≤0.05) due to the combination between filter methods and sperm fractions in progressive motility. Generally, filtration process improved (P≤0.05) sperm progressive motility than that before filtration. Higher sperm motility scores were found in semen fractions two and three (P≤0.05) than in semen fraction one and in the control sample (extended semen before filtration). Sephadex filter (67%), Sand filter (68%) and Swim-up (65%) method showed superior sperm motility scores than those recorded in control and other filters. While Albumin filter (fraction one) was higher in progressive motility than values in sperm fractions two, three and control samples, respectively.

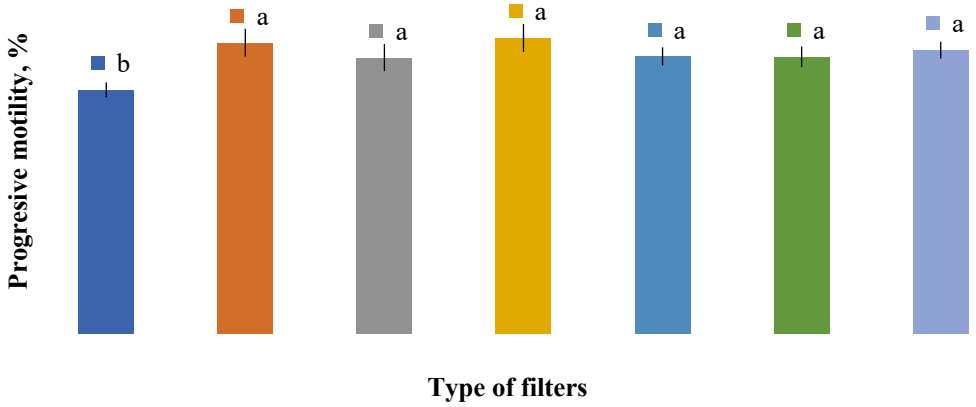

Figure (1): Overall percentage of progressive motility before and after filtration as affected by the type of filters (a, b shows differences between means at P≤0.05)

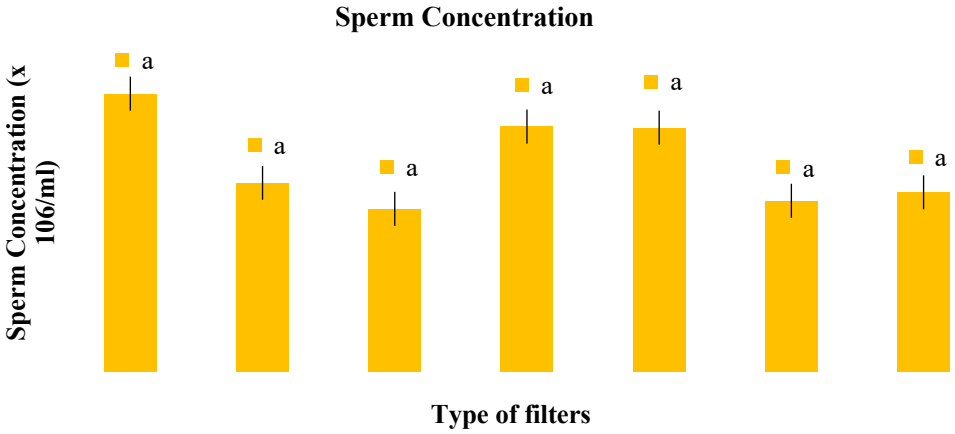

Figure (2): Overall concentration of rabbit's sperm (x10^6/ml) before and after filtration as affected by the type of filters (a,b shows differences between means at P≤0.05)

The effect of treatments on curvilinear velocity (VCL μm/s) of rabbit's sperm are presented in Figure (3). There are significant differences (P≤0.05) among sperm fractions and the interactions between treatments in sperm curvilinear velocity.

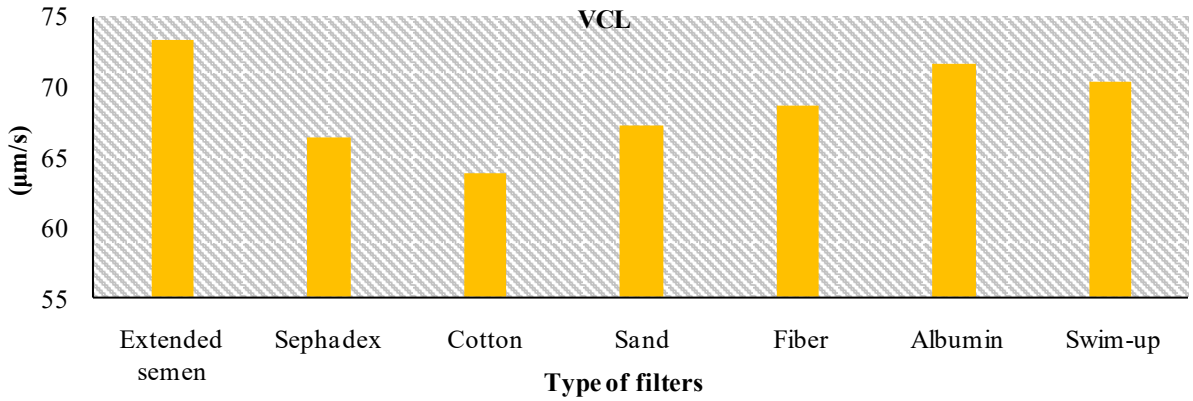

Figure (3): Overall Velocity Curvilinear (VCL µm/s) of rabbit's sperm before and after filtrations as affected by the type of filters.

Results presented Figure (4) showed the percentage of rabbit's live sperm before and after filtrations. The analysis of variance showed significant differences (P≤0.05) among the treatments and their interactions in percentage of rabbit's live sperm.

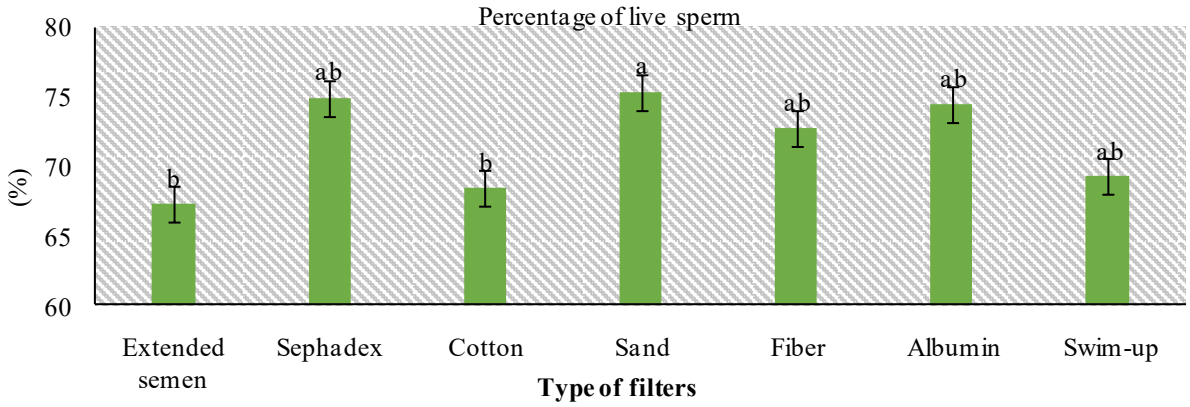

Figure (4): Overall percentage of rabbit's live sperm before and after filtrations as affected by the type of filters. (a, b shows differences between means at P≤0.05)

The effect of treatments on percentage of rabbit's sperm acrosome integrity are presented in Figure (5). The analysis of variance showed significant differences (P≤0.05) among the sperm fractions and the interactions between treatments in intactness of acrosome.

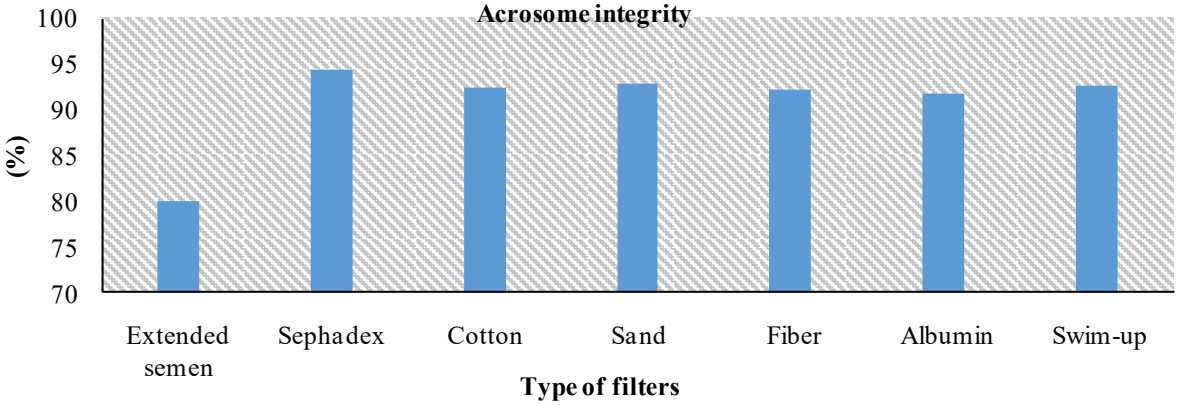

Figure (5): Overall percentage of rabbit's sperm acrosome integrity before and after filtrations as affected by the type of filters.

The percentage of morphologically normal rabbit's sperm as affected by treatments are presented in Figure (6). The analysis of variance showed significant differences (P≤0.05) due to the treatments and their interactions in percentage of morphologically normal forms of rabbit's sperm.

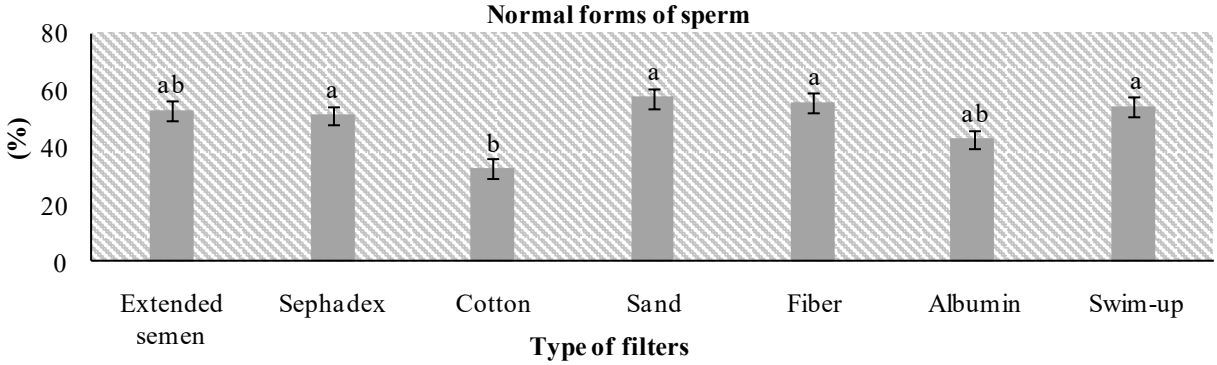

Figure (6): Overall percentage of morphologically normal forms of rabbit's sperm before and after filtrations as affected by the type of filters- (a, b shows differences between means at P≤0.05)

Results in Table (1) summarized the correlation coefficients among some studied traits. Results revealed that there were significant (P≤0.05) high positive correlations between progressive motility and acrosome integrity. High positive correlations were recorded between progressive motility and both the percentage of live sperm, and percentage of normal sperm. Between percentage of live sperm, acrosome integrity and percentage of normal sperm. Between percentage of live sperms and percentage of normal sperms. In contrast, there were significant (P≤0.05) high negative correlations between progressive motility and acrosome integrity.

Table (1): Correlation coefficients among some studied traits

	Live Sperms	Acrosome Integrity	Normal Sperms	Curvilinear Velocity
Progressive Motility	0.787	0.947*	0.812	-0.126
Live Sperm	1	0.599	0.592	0.507
Acrosome Integrity		1	0.664	-0.324
Normal Sperm			1	-0.256
Velocity Curvilinear				1

DISCUSSION

The present study was carried out to improve the semen quality of rabbits by removing dead, immotile and morphologically abnormal sperm by filtering ejaculated extended semen through five different filters Sephadex-G15, Albumin, Cotton, Synthetic Fiber, Sand and Sperm Swim-up procedure.

These results have been confirmed with several studies by Ayoub *et al.* (1996), Hammadeh *et al*. (2001), Henkel and Schill (2003), Januskauskas *et al.* (2005) and Lee *et al.* (2009), who reported that filtration techniques improved (P≤0.05) semen quality traits in farm animals compared with before filtration. These characteristics include progressive movement, morphologically normal sperm, viability, and acrosome-intact sperm. Moreover, the results recorded that Sand and Sephadex filters improved (P≤0.05) percentages of progressive motility and acrosome integrity compared to extended semen before filtration and other filters. Also, the highest percentages of morphologically normal sperm were recorded in Sand and synthetic Fiber filters (P≤0.05), but the lowest values were obtained in Swim-up and Cotton methods, respectively. The highest percentage of sperm viability was obtained in Sand filter (P≤0.05), but the lowest value was found in Cotton filter.

These results are similar with the results obtained by Ayoub *et al.* (1996), who found that Sephadex filter had higher sperm motility, live spermatozoa and acrosome integrity than Glass wool and Cotton filters in Boer goat semen. Ervandi (2013) reported that Albumin gradient improved semen quality compared to control samples in cattle sperm. Also, Grasa *et al.* (2004) found that the Swim-up procedure had higher sperm progressive motility, live spermatozoa and acrosome integrity than the row semen in ram. Also, Ahmad (2003) and Husna (2018) found that the Sephadex filter had higher sperm motility, live spermatozoa, and acrosome integrity than the row semen in buffalo. High positive correlations were recorded between

progressive motility and both the percentage of live sperm, and percentage of normal spermatozoa.

In the present study, sperm filtered by Sand were found to be the best of progressive motility and live sperm, followed by Sephadex-G15 filter compared with other filters. The percentage of motile sperm increasing after Sand or Sephadex filtration indicated that the trapping of immotile, abnormal, and dead spermatozoa in an effective way by physico-chemical reaction (Graham *et al.,* 1976; Ayoub *et al.,* 1996) or the appearance and bonding of specific protein on surface of capacitated spermatozoa (Samper, 1995) with the Sephadex particles. On the other hand, Fiber technique separated immotile sperm cells through densely packed fibers (Mortimer and Mortimer, 1992).

CONCLUSION

In general, the filtration process successfully maintained semen parameters to acceptable values recommended for artificial insemination in rabbits. The increase in most semen parameters was obtained by a significant degree of all used filtration methods compared to control. Even though, it is possible to successfully use all designed methods to eliminate dead and abnormal spermatozoa, the current results suggested that Sephadex-G15, Sand and Swim-up selection techniques could be more efficient to be practiced routinely in rabbit semen handling. Also, both second and third fractions of filtered semen could be recommended in commercial rabbit artificial insemination.

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خواص السائل المنوي للأرانب بعد الترشيح بفلاتر مصممة

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أجريت هذه الدراسة لتحسين جودة السائل المنوي للأرانب عن طريق إزالة الحيوانات المنوية الميتة والشاذة باستخدام خمس طرق ترشيح مختلفة وطريقة السباحة لأعلى. تم استخدام ستة طرق مختلفة لترشيح السائل المنوي (Sephadex-G15، الألبومين، القطن، الفايبر، والرمل) وتقنية سباحة الحيوانات المنوية لأعلي. تم استخدام 10 من ذكور الأرانب الشنشيلا الناضجة لجمع السائل المنوي. تم تقييم عينات السائل المنوي الخام قبل وبعد الفلترة من حيث الحركة والتركيز والVCLعن طريق تحليل حيوية الحيوانات المنوية بطريقة الCASA وتقدير سلامة الأكروسوم للحيوانات المنوية بصبغة الأكروسوم. أظهر تحليل التباين اختلافات معنوية ($P\leq0.05$) تعزى إلى الجمع بين طرق الفلترة وأخذ أكثر من راشح من عينات السائل المنوي، حسنت عملية الترشيح ($P\leq0.05$) الحركة التقدمية للحيوانات المنوية مقارنة بها قبل الترشيح. تم الوصول الي نسبة حيوية أعلى ($P\leq0.05$) للحيوانات المنوية في راشح السائل المنوي رقم 2 و 3 من تلك الموجودة في راشح السائل المنوي رقم 1 وفي العينة الكونترول. تم العثور على ارتباطات موجبة عالية بين معايير جودة السائل المنوي المدروسة. يمكن الاستنتاج أن معظم معايير جودة السائل المنوي قد تحسنت بشكل ملحوظ في جميع طرق الترشيح المستخدمة مقارنة مع العينات الكنترول (قبل الترشيح). يمكن أن تكون تقنيات فلاتر Sephadex-G15، الرمل والسباحة لأعلي أكثر فاعلية لتطبيقها بشكل روتيني في التعامل مع السائل المنوي للأرانب. أيضًا كما يمكن استخدام كلا الراشحين الثاني والثالث بشكل فعال في برامج التلقيح الصناعي.

الكلمات المفتاحية: الأرانب، السائل المنوي، الترشيح، فلاتر