Effect of Rabbit Semen Filtration Methods on Conception Rates and Altering Sex Ratio in Artificially Inseminated Rabbits

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Abstract: The present study was conducted to study the effect of filtering rabbit semen through five different filters: Sephadex-G15, Albumin, Cotton, Synthetic Fiber, Sand and Sperm swim-up method on conception rate of artificially inseminated rabbit does. Also, attempting to separate X-and Y-chromosome bearing spermatozoa by testing three semen fractions from each filter. Three methods were used to perform semen examinations: the 1st method was CASA to examine the filtered semen motility parameters, the 2nd method was Hoechst 33342 dye to stain the chromosomes to recognize X-and Y-spermatozoa and the 3rd method was determining sex ratio of bunnies born after insemination of rabbit does with selected fractions of filtered semen. Ten Chinchilla bucks and seventy-nine multiparous New Zealand White rabbit does were used in this study. Results clearly showed that semen filtration process and the selected semen fractions increased ($P<0.05$) percentages of sperm progressive motility, Y chromosome bearing spermatozoa, conception rate and male sex ratio of bunnies obtained than that before filtration. Conclusively, Sand, Albumin gradient, Fiber, Sephadex and Swim-up methods proved to be effective in improving semen quality and enabling sperm sexing which were accepted by Hoechst 33342 stain, CASA data and AI determinations of bunnies born in rabbits. These designed filters would enable researchers in this field to work in safe, less cost and effective methods to manage artificial insemination and reproduction in rabbit farms.

Keywords: Rabbits, semen filtration, semen sexing, artificial insemination

INTRODUCTION

Insemination with poor semen quality or even a double dose or more of low-quality semen seems inappropriate because dead spermatozoa have detrimental effects and negative toxic effects on the remaining normal sperm population (Lindemann *et al.,* 1982). The removal of dead and abnormal sperm of low-quality ejaculate is a more logical approach. Using sperm separating methods 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) were effective in improving sperm quality.

Nowadays, animal producers seek methods to fulfill the desire of consumers in the market especially the sex of the product they purchase. Based on the chromosomal content, spermatozoa are of two types, those bearing the X and Y chromosomes (Shettles, 1960; Gellatly, 2009). However, certain studies reported several morphological differences between the X and Y spermatozoa, most of the recent studies indicated that no major differences exist between the two types of spermatozoa (Hossain *et al.,* 2001; You *et al.,* 2017) except their DNA content. Separation of X- and Y-bearing spermatozoa for the purpose of pre-selection of the desired sex is very important in livestock production, which allows to produce the optimal proportion of females to males. The difference in genetic material (DNA) between the X and Y spermatozoa of domestic livestock is ranged between 3-4.2% (Hendriksen *et al.,* 1996). The DNA content as degree of differences varies from species to species and amounts to approximately 2.9% in human sperm (Johnson *et al.,* 1993), 3.8% in cattle (Garner *et al.,* 1983; Johnson and Welch, 1999), and as much as about 7.5% in chinchilla (Johnson, 1992). Several investigators have concluded that the variation in DNA content between X- and Y-spermatozoa may affect their motility and swimming pattern (Johnson, 1994). Researchers drawn two different hypotheses: (1) due to higher DNA content, X spermatozoa are more stable/viable than Y spermatozoa at least in the *in vitro* condition or (2) certain properties of Y cells may ensure that their prolonged viability in the female reproductive tract (*in vivo*) subsequently affects the lifespan of both cells in a distinct manner (Chen *et al.,* 2012; Carvalho *et al.,* 2013; You *et al.,* 2018). Controlling the sex ratio result is direct benefits in the livestock sector, faster genetic selection, animal welfare improvement, and lowering a bad environmental impact (De Canio *et al.,* 2014).

In addition to DNA content, other differences include the size (X-sperm > Y-sperm) (Cui and Matthews, 1993; Cui, 1997), surface charges on sperm (Y-sperm has a positive charge and X-sperm has a negative charge (Kiddy and Hafs, 1971) and cell surface antigens (Hoppe and Koo, 1984). It may be distinguished from X-sperm based on linearity and straightness of path. Based on the theoretical peculiarities, variety of methods has been reported for sorting X- and Y-sperm. These techniques include flow cytometry (Hendriksen, 1999), Percoll and Albumin gradient centrifugation (Koundouros and Verma, 2012), Swim up (Han *et al.,* 1993), Sephadex columns (Steeno and Justine, 2009), and H-Y antigen (Bennett and Boyse, 1973).

The present study aimed to study the effect of filtration methods on conception rates by improving semen quality of filtered rabbit semen. In addition, to compare the effects of different fractions of the filtration techniques on altering sex ratio of produced offspring.

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Therefore, the hypothesis of the current study assumed that the designed filters are successful in improving motility parameters, conception rate and to squeeze sex ratio to one sex direction in artificially inseminated rabbits.

MATERIALS AND METHODS

The current experiment was carried out at the Animal Production Department Laboratory and the rabbitry Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Six different semen filtration techniques were used to rabbit semen samples. Obtained filtered semen fractions were tested for X and Y chromosomes bearing spermatozoa by determining motility pattern with CASA method and Hoechst 33342 stain. In addition, to ensure and verify sexing data of semen fractions, an artificial insemination trial was obtained for rabbits with fractions from filtered semen. The filtration techniques used were: 1) Sephadex-G15, 2) Albumin gradient, 3) Sand, 4) Cotton, 5) Synthetic fiber, and 6) Sperm swim-up procedure.

Animals and husbandry

Ten Chinchilla bucks, aged 8-10 months were used for semen collection. Animals were healthy and free of any internal parasites or skin diseases. For the artificial insemination trial, seventy-nine multiparous New Zealand White does aged from 10-12 months were used. All animals were individually housed in galvanized wired cages, where feed and water were provided *ad libitum*. Animals were fed on basal pellet ration containing yellow corn, soybean meal, corn gluten, minerals and vitamins premix, bone meal, and molasses. The calculated chemical components of the diet were 17% crude protein, 2.8% fat, 10% crude fiber, and 2600 kcal digestible energy/kg. Semen samples were collected using a teaser female and artificial vagina. Collected semen samples with progressive motility over 65% were used throughout experiments.

Tris buffer preparation and dilution rate

Tris buffer was prepared by dissolving 3.605g tris, 2.024g citric acid, 1.490 g fructose in 100 ml distilled water. pH was adjusted to 6.8 with 1 M NaOH and/or 1 M HCL using ADWA pH meter. The fresh-extended semen samples were placed on top of the filtration columns and allowed for 15 min. Three fractions were taken from each filter after filtration and immediately evaluated for sperm motility.

Preparation of filters

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 gm) was compressed with the plunger to the bottom of the syringe to prevent loss of Sephadex. Sephadex was layered over the cotton and allowed to settle for 3 min. Semen samples were diluted five folds in a tris buffer before filtration. The extended semen was gently layered on to the column and filtered at room temperature (20-25°C). The syringes were upright placed in a holding stand for allowing the free drainage of fluid into the collecting tubes (Fayemi *et al.,* 1979). The columns were prepared immediately before filtration and kept in a vertical position at room temperature.

Sand. One gram of sand was sieved using 1.0 mm sieve and washed three times with distilled water and three 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 minutes and then three fractions were collected (1ml/fraction) and examined.

Synthetic fiber. 0.08 gm of soft synthetic fiber were put at the bottom of a plastic syringe and 3 ml of 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. According to the method that described by Ayoub *et al.* (1996), 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 (wetting step is required 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 (1 ml/fraction) and examined.

Swim-up technique. Eight ml of tris buffer placed in a 15 ml test tube in a 37° C water bath. 0.5 ml of semen were injected carefully at the bottom of the test tube and incubated for 1 h. 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. Computer-assisted semen analysis (CASA, Spermolab®, Cairo, Egypt) was used to evaluate motility pattern of diluted semen before and after filtration of semen samples. A drop of diluted semen (5 μL) was loaded into a pre-warmed slide, sample was allowed to settle on the mini-thermal warming stage (38°C). About 200 spermatozoa from 2-3 drops of each sample were evaluated for each specimen. The final analysis was done for each sample, including the following parameters: percentages of total sperm motility (TSM), progressive sperm motility (PSM).

Staining X- and Y-chromosomes bearing spermatozoa. Hoechst 33342 (bis Benzimide H 33342 tri hydrochloride, Sigma-Aldrich Chemical Co. ®875756-97-1) dye solution was prepared in distilled water (0.01 gm/ml) to make a concentrated stock solution and then added to the filtered semen, one drop of filtered semen (5 μL) was mixed with one drop of dye (5 μL) over a glass slide and cover slip was placed over the sample. The slides were incubated at 37°C for 30 minutes in the dark before being examined under a high power (1000X) fluorescent microscope (OLYMPUS BX43F). The X chromosome spermatozoa is larger than the Y chromosome spermatozoa, so it absorbed more of the dye and appeared brighter in the dark and Y-spermatozoa appeared dim under the fluorescent microscope. One hundred spermatozoa were counted to calculate the

percentage of X and Y spermatozoa before and after filtration.

Artificial insemination procedure. Based on the results of semen characteristics, four fractions high on progressive motility scores were selected to perform the artificial insemination. These fractions were: 1st fraction (10%) of Albumin gradient method, 3rd fraction (1hr) of Swim up method, 3rd fraction of the Sephadex method and 3rd fraction of the Sand method. Does were injected (i.m) with 0.2ml of Receptal® (Merck & Co., Inc.) immediately after the artificial insemination process. The pregnancy was diagnosed and confirmed on the 12th day from insemination. Checking for sex of offspring was performed 21 days after kindling.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS, 2003). Differences among means were detected using Duncan's new multiple test (Duncan, 1955). Pregnancy rates were transformed using arc sine for normalization. Correlation coefficients among traits were estimated. Two-ways analysis of variance was carried out for all traits using the following model:

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + e_{ijk}$$

Where:

Y_{ijk} = the observation on the k^{th} individual from the i^{th} methods of filtration in j^{th} semen fractions,

μ= the overall mean,

M_i = the fixed effect of the i^{th} methods of filtration,

F_i = the fixed effect of the i^{th} semen fractions,

MF_{ij} = the interaction between i^{th} methods of filtration and j^{th} semen fractions,

e_{ijk} = the random error associated with the ij^{kth} individual.

RESULTS

Results presented in Figure (1) show the percentage of progressive motility of rabbit's spermatozoa before and after filtration. The analysis of variance showed significant differences ($P \leq 0.05$) due to the combination between filter methods and sperm fractions in progressive motility. Filtration process improved ($P < 0.05$) sperm progressive motility than that before filtration. Higher sperm motility scores were found in semen fractions 2 and 3 ($P \leq 0.05$) than in semen fraction 1 and in the control sample (extended semen before filtration). Sephadex (67%) Sand filter (68%) and Swim-up (65%) method showed superior sperm motility scores than those recorded in control and other fractions. While Albumin filter sperm fraction one had higher 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

Results presented in Figure (2) show the percentage of rabbit's Y-chromosome bearing spermatozoa determined by Hoechst stain before and after filtration. The analysis of variance showed significant differences ($P \leq 0.05$) among treatments and their interactions in percentage of Y spermatozoa. The filtration process increased ($P \leq 0.05$) the percentage of Y spermatozoa obtained after filtration than that before filtration. However, the highest percentages of Y chromosome bearing spermatozoa were detected in Sand (60%) and Fiber (61%) filters, but the lowest value was recorded in Swim-up method (42%), irrespective of sperm fractions. Also, the highest percentages of Y chromosome bearing spermatozoa were recorded in sperm fractions two and three, but the lowest value was obtained in control sample, irrespective of filter methods. Moreover, sperm fraction number three in Sephadex and Sand filters showed a superior value in percentage of Y chromosome bearing spermatozoa percentage than those in control and other fractions.

Figure (2): Overall percentage of rabbit's Ychromo some bearing spermatozoa determined by Hoechst stain sperm before and after filtration as affected by type of filters

The percentage of rabbit's X chromosome bearing spermatozoa determined by Hoechst stain before and after filtrations are presented in Figure (3). The analysis of variance showed significant differences ($P \leq 0.05$) due to the treatments and their interactions in percentage of X chromosome bearing spermatozoa. The filtration process decreased ($P < 0.05$) the percentage of X chromosome bearing spermatozoa than that before filtration. Lower percentages of X chromosome bearing spermatozoa were found in Sand (40%) and Fiber (38%) filters than those recorded in other filters and control sample, irrespective of sperm fractions. Also, lower percentage of X chromosome bearing spermatozoa were found in fractions two and three than that in fraction one and control sample. Sperm fraction number three in Sephadex and Sand filters were lower in the percentage of X chromosome bearing spermatozoa than those recorded in control and other sperm fractions. On the other hand, fraction three in Swim-up method was significantly higher in the percentage of X chromosome bearing spermatozoa count than those recorded in control and other sperm fractions.

The effect of treatments on does conception rate and bunnies sex ratio are presented in Table (1). The analysis of variance showed significant differences ($P \leq 0.05$) due to filter type in bunnies sex ratio. The filtration process improved non significantly the conception rate and squeezed ($P \leq 0.05$) sex ratio to one sex direction than that before filtration. Higher conception rates were recorded in fraction three in both Sand (66%) and Swim-up filters (66%) ($P \leq 0.05$) than those recorded in control and other filter types. Sperm fraction number three in Sand and one in Albumin showed a superior total litter size than those in control and other filters. Higher percentages of male sex ratio were obtained with sperm fraction three in Sephadex, Sand and number one in Albumin methods ($P \leq 0.05$) than in control and other filters and fraction. The opposite trend of these results was obtained in the percentages of female sex ratio with fraction three in Swim-up method.

Figure (3): Percentage of rabbit's X chromosome bearing spermatozoa determined by Hoechst stain sperm before and after filtration as affected by type of filters

Table (1): Conception rate of does and sex ratio of bunnies as affected by treatments (Mean+ S.E)

Method of filtration	No of Does	Conception rate	Total litter size	Sex Ratio	
				Males (%)	Females (%)
Control(Natural mating)	15	53.33±13.33	47	49.13±4.29 ^{b}	50.87±4.29 ^{b}
Control (AI by extended semen before filtration, at zero time)	8	50.03±18.89	32	48.50±4.34 ^{b}	51.5±4.34 ^{b}
Sephadex (3rdFraction)	13	53.85±14.39	48	64.02±3.35 ^{a}	36.05±3.35 ^{c}
Sand (3rdFraction)	13	66.67±14.21	50	69.25±3.17 ^{a}	30.75±3.17 ^{c}
Albumin(1stFraction)	18	55.56±12.05	66	68.50±4.50^{a}	31.70±4.50^{c}
Swim-up(3rdFraction)	12	66.67±14.21	48	33.25±2.30^{c}	66.75±2.30^{a}

a, b Means within the rows and columns with different superscripts are significantly different (P≤0.05)

Correlation coefficients among some studied traits

Results in Table (2) summarized the correlation coefficients among some studied traits. Results revealed that there was significant (P≤0.05) high positive correlation between progressive motility and percentage of Y chromosome bearing spermatozoa. However, high positive correlation was recorded between progressive motility and conception rate. Also, between percentage of Y chromosome bearing spermatozoa and conception rate. In contrast, there were significant (P≤0.05) high negative correlations between percentage of X chromosome bearing spermatozoa and progressive motility, percentage of Y chromosome bearing spermatozoa and conception rate.

Table (2): Correlation coefficients among some studied traits

	Conception rate	Y- Chromosome Bearing Spermatozoa	X-Chromosome Bearing Spermatozoa
Progressive motility	0.873	0.978*	-0.986*
Conception rate	1	0.868	-0.887
Y- Chromosome Bearing Spermatozoa		1	-0.999**

DISCUSSION

Results of the present study all filtration techniques improved (P≤0.05) sperm progressive motility, conception rate and squeezed sex ratio to one sex direction compared with before filtration semen. Conception rates were higher (P≤0.05) in Sand filter and Swim-up method at sperm fraction three compared with other methods and extended semen before filtration.

Results showed high positive correlations were recorded between conception rate and progressive motility. These results agreed with previous reports by Ahmad *et al.* (2003), who found that the Sephadex filter improved conception rates and percentage of pregnancy compared to the row semen in buffalo. Also, Sieme *et al.* (2003) reported that percentage of pregnancy improved after filtration with glass wool in mares compared to control samples. Also, Ren *et al.* (2004)found that percentage of pregnancy improved after filtration with Albumin gradient compared to control samples in human. These results are in agreement with previous reports by Rurangwa *et al.* (2004), who found that conception rate or fertilization capability of sperm is highly correlated with sperm concentration, and progressive motility.

In the current work, rabbit semen filtered by Sand method was found to be the best in progressive motility, followed by Sephadex G15 filter compared with other filters. Regardless to the natural sex ratio during spermatogenesis is expected to be 50:50 (Umehara *et al.,* 2019), our results revealed a change in the ratio of the Y and X chromosome bearing spermatozoa by semen filtration methods. Filtration process by Sand and Fiber increased (P≤0.05) percentages of Y spermatozoa in filtrates than that before filtration and the lowest values were obtained in Swim-up and Cotton methods. However, 2nd and 3rdsperm fractions increased (P≤0.05) percentages of Y chromosome bearing spermatozoa compared with sperm 1stfraction and extended semen before filtration. Also, the percentage of male sex ratio of offspring was increased significantly (P≤0.05) in Sand and Sephadex filters in sperm fraction three and Albumin method in sperm fraction one compared with extended semen before filtration and other methods. Moreover, a highly significant (P≤0.01) positive correlation was detected between percentage of progressive motility and Y chromosome bearing spermatozoa. These results are similar with that recorded by Galarza *et al.* (2018), who reported that Sephadex filtration enriched the Y

chromosome in ram semen. Also, Naniwa and Uchiyama (2021) reported that Albumin gradient increased ratio of the Y chromosome in cattle semen. An effective method of sex preselection is required depending on the small difference in DNA content that exists between X- and Y-chromosome bearing sperm. Since the first report by Johnson *et al.* (1989) in rabbits, flow cytometric sorting of X- and Y-sperm based on the difference in DNA content combined with artificial insemination and in vitro fertilization, has been shown to be an effective and promising method to preselect the sex of newborn (Maxwell *et al.,* 2004).

More reliable techniques have been used to evaluate the true chromosomal content of sperm cells after separation into X and Y chromosome-bearing spermatozoa such as quinacrine mustard staining, fluorescence in situ hybridization (FISH) (Rose and Wong, 1998), nested PCR and the latest was quantitative real time PCR (Parati *et al.,* 2006). The efficiency of separation was determined based on the counted percentages of X- and Y chromosomes.

In the present study, the efficiency of the separation procedure is based on two different testing methods. The first was a microscopic method by staining the filtered sperm with synthetic fluorescent bis-benzimidazole dye Hoechst 33342. The second method was in-vivo, by artificial insemination of rabbits with filtered semen. Both methods illustrated the full picture and approved the hypothesis of altering sex ratio of sperm after filtration. Sexed semen has brought a revolution to the animal production industry and has successfully been used in cattle (Seidel, 1999). Due to the biological and economic disadvantages of flow cytometry, some efforts have been made to develop alternate sperm sexing methods based on density and motility of X and Y chromosome-bearing sperm. Among these, the modified Swim-up method (Azizeddin *et al.,* 2014) was capable to separate X and Y chromosome bearing sperm, as X chromosome bearing sperm has more DNA content than Y chromosome bearing sperm which results in faster migration velocity of Y chromosome bearing sperm than the X chromosome bearing sperm (Yan *et al.,* 2006).Results of the present study ensured that Swim-up method did not compromise the sperm quality parameters (plasma membrane and acrosomal integrity); rather it improved the sperm quality (progressive motility) for X and Y chromosome sorted fractions compared to unsorted control samples.

In this context, the discontinuous Albumin gradient technique has generated huge contradictory evidence since the mechanisms of Y-chromosome bearing spermatozoa enrichment and was not proven experimentally by some authors (Ericsson *et al.,* 1973; Claassens *et al.,* 1995). In the present study, the Albumin gradient technique was performed to separate rabbit semen into X- and Y-chromosome bearing spermatozoa as a modification of Ericsson *et al.* (1973) method who reported that the enriched population of Y-chromosome bearing spermatozoa could be discovered by using bovine Albumin density gradient. The Albumin gradient used in the present study was a three-phase gradient (4% over 6% over 10% BSA), the technique performed in this study was simpler as compared to the Ericsson *et al.* (1973) method, whereby the washing and centrifugation steps in the former were skipped to minimize pressure and reduce sperm mortality. The efficiency of separation was determined based on the counted percentages of X- and Y-chromosomes by staining with Hoechst dye for each individual sample. On the other hand, Mahadevan and Baker (1984) were the first to describe the Swim-up method as an effective way of separating spermatozoa. The Swim-up technique has previously been reported to yield sperm population which resulted in a high percentage of male births (Check *et al.,* 1989). The present study reported higher recovery rates of motile sperm. The improved quality of X-sperm might be due to the location of X-sperm fractions in lower layers compared to upper layers of Y-sperm fractions. The results of this study are in line with (Ericsson, 1994) who reported that sperm recovery and quality in the final fraction is affected by many factors including temperature, solution, and the isolated tube dimensions. It might be speculated that X-sperm, owing to their higher density due to the size of the sperm head (Cui and Matthews, 1993), move out from semen samples in higher percentage (Hafez, 1991) and travel to upper (1 mL) layers compared to distant (4-5 mL) travel by Y-sperm in a long narrow glass tube. It is also relevant to mention that Y-spermatozoa have higher forward velocity than X-spermatozoa (Ericsson *et al.,* 1973). Here we can hypothesize that Swim-up is more efficient for the selection of X-sperm that might have further implications in the dairy industry if supported by extensive fertility trials. However, in our study, the Swim-up method generated a higher proportion of X-sperm in the third fraction and Y chromosome-bearing sperm in first fraction without compromising semen quality. Finally, the present study has suggested the advantages of using simple, inexpensive, and less deleterious semen filtration methods to improve sperm quality and sexing spermatozoa. Moreover, the artificial insemination trial approved the successful sperm separating methods through improving sperm motility, conception rates, and directing sex of offspring to one direction.

CONCLUSION

Sand, Albumin gradient, Fiber, Sephadex filters and Swim-up method proved to be effective in improving sperm progressive motility and sperm sexing as accepted by Hoechst 33342 stain, CASA and AI results through squeezing sex of newborn to one direction in rabbits. The designed semen filtering methods will enable researchers to work in a safe, and less-cost effective way in semen handling and sperm sexing. Further studies are suggested to control sexing ratio of bunnies by inventing modified

protocols, employing simpler and less expensive equipment and increase number of inseminated does

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تأثير طرق ترشيح السائل المنوي على معدلات الحمل وتغيير النسبة الجنسية في الأرانب الملقحة صناعيا

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أجريت الدراسة الحالية لدراسة تأثير ترشيح السائل المنوي للأرانب من خلال خمسة فلاتر مختلفة:السيفادكس، الألبيومين، الألياف الصناعية، والرمل وطريقة سباحة الحيوانات المنوية لأعلى على معدلات الحمل في الأرانب الملقحة صناعيا. كذلك محاولة فصل الحيوانات المنوية الحاملة للكروموسوم Xو Yعن طريق اختبار ثلاثة أجزاء من مرتشحات السائل المنوي لكل فلتر. تم استخدام ثلاث طرق لإجراء فحوصات السائل المنوي: الطريقة الأولى كانت CASA لفحص معايير حيوية السائل المنوي المرشح، والطريقة الثانية كانت صبغة Hoechst 33342 لصبغ الكروموسومات للتعرف على الحيوانات المنوية X و Y والطريقة الثالثة كانت تحديد النسبة الجنسية للمواليد في الأرانب الملقحة صناعياً بالسائل المنوي المرشح. تم استخدام عشرة ذكور من نوع الشينشيلا وتسعة وسبعين أنثي من سلالة نيوزيلندي الأبيض. أظهرت النتائج بوضوح أن عملية ترشيح السائل المنوي وأجزاء السائل المنوي المرشحة أدت إلى زيادة ($P<0.05$) في نسب الحركة التقدمية للحيوانات المنوية، ونسبة الحيوانات المنوية الحاملة للكروموسوم Y،وارتفاع معدل الحمل وزيادة نسبة الذكور في الخلفة مقارنة بتلك التي كانت قبل الترشيح. إجمالا أثبتت طرق الترشيح بالرمل والألبومين والألياف و Sephadex و Swim-up فعاليتها في تحسين جودة السائل المنوي والقدرة على تجنيس الحيوانات المنوية والتي ثبتت صحتها من خلال نتائج صبغة Hoechst 33342، CASA و نتائج التلقيح الصناعي من الصغار المولودة في الأرانب، مما يتيح للباحثين في هذا المجال العمل بأمان وبأقل تكلفة للطرق للتحكم في التناسل بمزارع الأرانب.

الكلمات المفتاحية: الأرانب، ترشيح السائل المنوي، تجنيس السائل المنوي، التلقيح الاصطناعي