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SEMEN QUALITY, FERTILITY RATE AND SEX RATIO OF THE CENTRIFUGED COOLED RABBIT SPERMATOZOA

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Twenty bucks and two–hundred eight multiparous lactating New – Zealand White (NZW) does rabbit, were used in the present study. Semen was collected and divided into six equal portions. The first portion was kept as a control (without centrifugation). The second, third, fourth, fifth and sixth portions were centrifuged through Percoll solution at 250, 500, 750, 1000 and 1250 xg for 15 minutes, respectively. The sperm plugs were resuspended to a final concentration of 60 x 10^6 sperm/ml in sucrose- yolk-citrate extender. The extended semen samples were then stored at 5ºC for 3 days. After each storage time (0, 1, 2 and 3 days), semen quality and enzymatic activity were estimated. Fertility rate and sex ratio of the does artificially inseminated with the centrifuged and non- centrifuged semen, were also assessed.

The obtained results showed that the percentage of motile spermatozoa increased significantly (P<0.01), while the percentages of dead spermatozoa, sperm abnormalities, acrosomal damage of spermatozoa and leakage of aspartate- aminotransferase (AST) and alanine – aminotransferase (ALT) enzymes into the extracellular medium were significantly (P<0.01) decreased with centrifuged semen as compared to the non- centrifuged one. The highest (P<0.01) value of sperm motility and the lowest (P<0.01) values of dead spermatozoa, sperm abnormalities, acrosomal damage of spermatozoa and amounts of AST and ALT enzymes were recorded with the centrifuged semen at 500 and 750 xg for 15 minutes. The percentage of sperm motility decreased significantly (P<0.01), while the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa and amounts of AST and ALT enzymes were increased significantly (P<0.01) with the increase in of storage times at 5ºC for up to 3 days in both centrifuged and non-centrifuged rabbit semen. The fertility rates and total number of kits at birth were not significantly different in does artificially inseminated with the centrifuged than in those with the non-centrifuged semen. Centrifugation of semen at 500 and 750 xg insignificantly increased the fertility rate than at 250, 1000 and 1250

xg for 15 minutes. However, centrifugation of semen before artificial insemination increased significantly (P<0.05) the proportion of females kits and decreased significantly (P<0.05) the proportion of the male kits as compared to the non-centrifuged semen.

Keywords: Fertility, Percoll gradient centrifugation, semen quality, sex ratio.

In animal production systems, the concept of being able to alter the sex ratio is attractive. With increased output of animal protein being necessary to help feed a globally expanding human population, the increased growth rate and more efficient production of lean meat by male animals would make pre-selection desirable.

Low concentrations of spermatozoa in some rabbit ejaculates necessitate removal of seminal plasma by centrifugation to maintain desired insemination volume (Rombe *et al*., 1965). Dilution of semen or removal of seminal plasma is believed to be necessary for prolonged storage (Roca *et al.*, 1997).

Separation of mammalian semen into X–and Y-chromosome bearing fractions, and the use of such semen in an artificial insemination programme, would appear to be the most elegant, rapid and efficient way of ensuring an altered sex ratio. Application of sexed semen for production of livestock of predetermined sex is dependent on economics, effectiveness and ease of use. The most cost effective approach for achieving sex pre-selection would involve separation adequate numbers of the X from the Y sperm followed by use of the individual sperm populations for artificial insemination.

Since the 1970,s several investigators have made attempts to separate X and Y bearing spermatozoa by mans of various gradient techniques, such as discontinuous albumin gradients (Ericsson *et al.*, 1973), sephadex columns (Steeno *et al.,* 1975), discontinuous Percoll gradients (Lizuka *et al.,* 1987) or the swim-up procedure (Check and Katsoff, 1993). None of these methods have been successful in achieving a precise separation of X – and Y-bearing spermatozoa and only flow cytometry has proven to be effective (Garner *et al.,* 1983). Flow cytometrically sex-sorted semen is not yet applicable to commercial rabbit farms since technology costs make it incompatible with the narrow financial profits generated by this species. Of the other separation methods mentioned above, discontinuous Percoll gradient was the only technique that significantly altered the X and Y- bearing sperm ratio, although the degree of enrichment was small (Kobayashi *et al.,* 2004) .

The purpose of the present study was to explore the possibility of modifying the sex ratio in rabbit litters obtained by artificial insemination using practical method applicable (Percoll gradient technique) to the working

routine of commercial rabbit farms. Semen quality, enzymatic activities and fertility rate as affected by removal of seminal plasma using centrifugation had also been assessed.

MATERIALS AND METHODS

The present study was carried out in the Rabbit Farm, Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, and in a Private Rabbit Farm, Tarout Village, Zagazig City, Sharkiya Province.

Twenty bucks and two hundred eight multiparous lactating New Zealand White (NZW) does rabbit of approximately 40 weeks of age and 3.0 – 3.5 kg in body weight were used.

The animals were healthy and clinically free of external and internal parasites and were raised in flat deck batteries with universal specifications. The batteries were supplied with feeders and automatic fresh water drinkers and were efficient for hygienic control. All batteries were located in naturally ventilated windowed house. Environmental and feeding conditions, as well as, reproductive management of the rabbit were as previously described by Quintela *et al*. (2004).

Semen was collected twice weekly for 10 weeks by means of an artificial vagina and immediately evaluated and pooled after collection. Only ejaculates having 60% progressive sperm motility were used. Semen was divided into six equal aliquots. In the first aliquot, semen was non-centrifuged (control). The second, third, fourth, fifth and sixth aliquots were centrifuged (treated) at 250 , 500, 750, 1000 and 1250 *xg* for 15minutes through Percoll gradient according to Vega *et al.* (2007). Percoll gradients (Pharmacia Fine Chemicals, Upssala, Sweden) were made by mixing the appropriate volumes of Percoll and phosphate Buffer Saline PBS to obtain 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90% Percoll solutions. Each 1 ml of the Percoll solutions was consecutively layer in a 15-ml falcon tube (90% Percoll solution at the bottom of the tube and 40% at the upper layer) and then 1 ml of the sperm suspension was overlaid on the 40% Percoll solution. The seminal plasma was removed and sperm plugs were re-suspended in citrate – based – diluents to a volume equal to that of the semen before centrifugation. The extender contained 2.90 gm sodium citrate, 1.25 gm sucrose, 0.04 gm citric acid, 10 ml egg yolk, 500 µg streptomycin and 500 IU penicillin /100ml as described by Evans and Maxwell (1987). The supernatant was discarded and the sperm plugs were re-suspended to a final concentration of $60x10^6$ spermatozoa / ml with an extension rate of 1 semen : 6 extender. Each portion of the diluted centrifuged or non-centrifuged semen was then cooled slowly to 5ºC over 2 hours and then stored at this temperature for up to 3 days. After each storage time (0, 1, 2

and 3 days), percentages of sperm motility, dead spermatozoa, sperm abnormalities and acrosomal damage were recorded according to Watson (1975). Semen samples (centrifuged and non-centrifuged) were also centrifuged at 600 *xg* for 15 minutes and the supernatant was removed and stored at -20˚C untill enzymatic assay. Aspartate–aminotransferase (AST) and alanine– aminotransferase (ALT) enzymes were determined according to Ritman and Frankle (1957).

In the fertility trial, ovulation was induced in does by injecting 20µg (1ml) gonadorelin intramuscularly (Induced GnRH), Ovejero, Leon, Spain) immediately after artificial insemination with 1 ml containing 60 X 10^6 spermatozoa (Boussit, 1989). Rabbit does were palpated to establish pregnancy after 14 days of insemination. The fertility rates of the does rabbit artificially inseminated with the centrifuged and non-centrifuged spermatozoa, were also assessed.

Data were statistically examined by the analysis of variance according to Snedecor and Cochran (1982). The significant differences between means were evaluated by Duncans New Multiple Range test (Duncan, 1955). Fertility rate results were analyzed by Chi – square test.

RESULTS AND DISCUSSION

Semen quality:

Table 1 showed that the percentages of sperm motility were significantly (P<0.01) higher after reactivation by centrifugation of rabbit semen than in non-centrifuged semen. The percentage of sperm motility was significantly (P<0.01) higher in centrifuged semen at 500 or 750 *xg* for 15 minutes than in those at 250, 1000 and 1250 *xg* for 15 minutes or non-centrifuged spermatozoa. The highest (P<0.01) value of sperm motility was recorded after centrifugation at 750 *xg* and the lowest (P<0.01) value was recorded at 250 *xg* for 15 minutes or non- centrifuged spermatozoa. Parrish *et al.* (1995) found that centrifugation of semen on the Percoll gradient for 15 minutes at 700 *xg* was sufficient to obtain optimal recovery of motile spermatozoa. In human, where few spermatozoa may be motile or a high number of abnormal spermatozoa may be present, the separation of spermatozoa by normal morphology may be of a primary importance (Ng *et al.*, 1992). Corteel (1975) found that low molecular weight component was retained with spermatozoa and the high molecular weight fraction was removed by centrifugation. Jasko *et al.* (1991) also confirmed that high molecular weight fractions in seminal plasma depress sperm motility, livability and absolute index of livability. El-Kelawy (2002) showed that centrifugation of rabbit semen on Percoll gradient at 600 – 1200 r.p.m for 15 minutes was significantly increased of sperm motility. Zeidan *et al*. (2004), also found that the highest values of sperm motility was recorded in the

Table 1. Mean percentage of sperm motility of the non- centrifuged and centrifuged rabbit semen, during storage at 5 ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	67.25 [a] ±2.01	69.32 [a] ±1.76	71.92 [a] ±1.45	73.12 [a] ±1.65	71.18 [a] ±2.28	71.13 [a] ±2.13
1	50.22 [b] ±1.82	61.42 [b] ±2.38	67.18 [b] ±1.82	67.18 [b] ±1.28	66.24 [b] ±1.16	64.13 [b] ±1.35
2	39.52 [c] ±2.10	52.63 [c] ±1.46	55.16 [c] ±1.90	58.25 [c] ±2.13	53.42 [c] ±2.08	52.54 [c] ±1.72
3	26.14 [d] ±1.42	34.16 [d] ±1.28	40.35 [d] ±1.17	42.36 [d] ±1.14	40.38 [d] ±1.16	35.19 [d] ±1.18
Means	**45.78 [E] ±1.72**	**54.64 [D] ±1.48**	**58.65 [AB] ±1.39**	**60.23 [A] ±1.32**	**57.81 [BC] ±1.37**	**55.76 [C] ±1.55**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-E: Means with different superscripts in the same row, differ significantly (P<0.01).

centrifuged semen at 950 *xg* for 15 min., in goats semen. Viability of spermatozoa reduction with increasing centrifugation might be caused by a direct effect of centrifugation on the sperm plasma membrane resulting in loss of intracellular components such as nucleotides and nicotamide dinucleotides **(**Watson *et al.,* 1992). This damage may increase during centrifugation and washing, which are required for sperm selection (Schill and Walff, 1974 and Sanchez *et al.,* 1995).

Data presented in Tables 2, 3 and 4 show that the effect of type of rabbit spermatozoa (centrifuged or non- centrifuged) on percentages of dead spermatozoa, sperm abnormalities and acrosomal damage were highly significant (P<0.01). The percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa after centrifugation at different forces (250, 500, 750, 1000 and 1250 *xg* for 15 min) were significantly (P<0.01) lower than in non-centrifuged semen. The lowest (P<0.01) values of dead spermatozoa, sperm abnormalities and acrosomal damage were recorded after centrifugation of semen at 500 and 750 *xg* for 15 min, and the highest (P<0.01) values were recorded after centrifugation at 250 *xg* for 15 min and non-centrifuged semen. Martinus *et al.* (1991) reported that the presence of seminal plasma caused increase in release of the amino acid oxidase, an enzyme responsible for reduction in sperm motility, resulting in increase of dead and abnormal spermatozoa. In addition, the removal of seminal plasma by washing of spermatozoa before dilution improved the survival rate of cells during preservation (Memon *et al*., 1985 and Roca *et al.,* 1997). The presence of phosphalipase A (egg yolk coagulating enzyme) in seminal plasma reduced the survival rates of spermatozoa diluted and stored at low temperature in egg yolk extender (Roy, 1957).

Table 2. Mean percentage of dead spermatozoa of the non – centrifuged rabbit semen, during storage at 5ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	21.16 ^{d} ±1.03	19.15 ^{c} ±0.75	16.12 ^{c} ±0.81	13.41 ^{c} ±0.65	15.32 ^{c} ±0.84	16.24 ^{d} ±1.18
1	23.17 ^{c} ±1.12	20.18 ^{c} ±1.15	17.08 ^{c} ±1.02	14.18 ^{c} ±0.76	16.27 ^{c} ±0.96	19.08 ^{c} ±0.92
2	28.19 ^{b} ±1.19	23.10 ^{b} ±1.08	20.12 ^{b} ±0.78	16.22 ^{b} ±0.81	22.14 ^{b} ±1.03	23.11 ^{b} ±1.13
3	35.28 ^{a} ±1.27	28.31 ^{a} ±1.01	24.13 ^{a} ±1.03	19.11 ^{a} ±1.02	27.15 ^{a} ±0.88	29.12 ^{a} ±0.95
Means	**26.95 ^{A} ±1.75**	**22.69 ^{B} ±1.14**	**19.36 ^{C} ±1.00**	**15.73 ^{D} ±0.71**	**20.22 ^{BC} ±1.53**	**21.89 ^{BC} ±1.55**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-D: Means with different superscripts in the same row, differ significantly (P<0.01).

Table 3. Mean percentage of abnormal spermatozoa of the non-centrifuged rabbit semen, during storage at 5ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	16.25 ^{d} ±0.84	14.02 ^{d} ±0.81	11.13 ^{d} ±0.61	7.65 ^{d} ±0.28	12.81 ^{d} ±0.42	13.25 ^{d} ±0.22
1	19.18 ^{c} ±0.85	15.25 ^{c} ±0.92	12.28 ^{c} ±0.45	8.12 ^{c} ±0.92	13.75 ^{c} ±0.65	14.16 ^{c} ±0.41
2	23.41 ^{b} ±1.08	18.13 ^{b} ±0.86	14.17 ^{b} ±0.64	11.09 ^{b} ±0.81	15.42 ^{b} ±0.71	17.31 ^{b} ±0.91
3	30.32 ^{a} ±1.12	22.18 ^{a} ±1.02	19.26 ^{a} ±0.81	12.13 ^{a} ±1.08	22.89 ^{a} ±0.82	22.19 ^{a} ±0.88
Means	**22.29 ^{A} ±1.70**	**17.40 ^{B} ±1.01**	**14.21 ^{C} ±1.00**	**9.75 ^{D} ±0.61**	**16.22 ^{BC} ±1.27**	**16.73 ^{BC} ±1.12**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-D: Means with different superscripts in the same row, differ significantly (P<0.01).

The prolongation of storage time at 5ºC decreased significantly (P<0.01) the percentage of sperm motility, while percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of rabbit spermatozoa centrifuged at different forces or non-centrifuged spermatozoa increased significantly (P<0.01). Similar trends was reported by Memon *et al.* (1985), Roca *et al.* (1997) and Zeidan *et al.* (2004).

Enzymatic activities:

As shown in Tables 5 and 6, centrifugation of rabbit semen at different forces (250, 500, 750, 1000 and 1250 *xg*) for 15 minutes significantly (P<0.01) decreased the amount of AST and ALT enzymes

Table 4. Mean percentage of acrosomal damage of spermatozoa of the non centrifuged rabbit semen, during storage at 5ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	11.75 ^{d} ±0.75	8.11 ^{d} ±0.41	6.13 ^{d} ±0.31	4.08 ^{d} ±0.16	5.15 ^{d} ±0.15	7.05 ^{d} ±0.21
1	13.18 ^{c} ±0.82	9.13 ^{c} ±0.38	7.12 ^{c} ±0.25	4.13 ^{c} ±0.21	7.12 ^{c} ±0.18	9.12 ^{c} ±0.08
2	19.25 ^{b} ±0.48	11.25 ^{b} ±0.71	9.08 ^{b} ±0.18	6.15 ^{b} ±0.25	10.25 ^{b} ±0.36	11.14 ^{b} ±0.18
3	26.48 ^{a} ±0.81	14.19 ^{a} ±0.92	13.15 ^{a} ±0.17	9.22 ^{a} ±0.18	15.17 ^{a} ±0.29	15.27 ^{a} ±0.42
Means	**17.67 ^{A} ±1.87**	**10.67 ^{B} ±0.75**	**8.87 ^{C} ±0.86**	**5.65 ^{D} ±0.55**	**9.42 ^{BC} ±1.21**	**10.65 ^{BC} ±0.97**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-D: Means with different superscripts in the same row, differ significantly (P<0.01).

Table 5. Aspartate aminotransferase (11/10^{6} spermatozoa) enzyme activity of the non- centrifuged and centrifuged rabbit semen during storage at 5ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	61.18 ±2.11^{d}	42.19 ^{d} ±1.17	40.65 ^{d} ±1.52	35.48 ^{d} ±1.25	40.16 ^{d} ±1.12	52.12 ^{d} ±1.25
1	68.22 ±1.82^{c}	48.11 ^{c} ±1.38	43.50 ^{c} ±1.38	39.72 ^{c} ±1.18	43.20 ^{c} ±2.13	58.13 ^{c} ±1.31
2	82.44 ±1.65^{b}	56.13 ^{b} ±2.15	50.38 ^{b} ±2.14	46.52 ^{b} ±1.67	54.35 ^{b} ±1.17	65.18 ^{b} ±1.52
3	90.16 ±2.13^{a}	67.18 ^{a} ±2.16	58.25 ^{a} ±1.82	54.81 ^{a} ±1.72	61.18 ^{a} ±1.22	74.14 ^{a} ±2.18
Means	**75.49 ^{A} ±1.30**	**53.40 ^{C} ±1.07**	**48.19 ^{D} ±0.78**	**44.38 ^{E} ±0.80**	**49.72 ^{D} ±0.97**	**62.39 ^{B} ±0.94**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-E: Means with different superscripts in the same row, differ significantly (P<0.01).

released into the extracellular medium than in non-centrifuged semen throughout the storage period which lasted, as long as 3 days. The highest (P<0.01) amounts of AST and ALT enzymes released into the extracellular medium were recorded with the semen after centrifugation at 1250 *xg* for 15 minutes and non- centrifuged semen and the lowest (P<0.01) amounts were recorded with the semen after centrifugation at 1000 *xg* for 15 minutes. These results were in agreement with those of Goyal *et al.* (1996) in buffalo, El-Kelawy (2002) in rabbit and Zeidan *et al*. (2004) in goat spermatozoa. These results may be due to the removal of the inhibitory factors found in seminal

Table 6. Alanine – aminotransferase (11/10^6 spermatozoa) enzyme activity of the non- centrifuged and centrifuged rabbit semen during storage at 5ºC for up to 3 days.

Storage time (Days)	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
0	12.41 ^{d} ±0.53	7.25 ^{d} ±0.72	5.25 ^{d} ±0.32	5.17 ^{d} ±0.28	6.16 ^{d} ±0.31	10.72 ^{d} ±0.71
1	15.72 ^{c} ±0.81	9.72 ^{c} ±0.42	7.82 ^{c} ±0.48	6.35 ^{c} ±0.66	9.22 ^{c} ±0.82	13.52 ^{c} ±0.48
2	19.82 ^{b} ±0.72	13.85 ^{b} ±0.38	11.13 ^{b} ±0.82	8.19 ^{b} ±0.42	12.51 ^{b} ±0.55	16.48 ^{b} ±1.02
3	25.31 ^{a} ±1.02	20.16 ^{a} ±0.52	16.28 ^{a} ±0.57	13.46 ^{a} ±0.72	16.38 ^{a} ±0.73	24.19 ^{a} ±1.11
Means	**18.32 ^{A} ±0.50**	**12.75 ^{C} ±0.56**	**10.12 ^{D} ±0.47**	**8.29 ^{E} ±0.36**	**11.07 ^{D} ±0.43**	**16.23 ^{B} ±0.57**

a-d: Means with different superscripts in the same column, differ significantly (P<0.01).
A-E: Means with different superscripts in the same row, differ significantly (P<0.01).

plasma which caused cell membrane damage and increased AST and ALT release (Sengupta *et al.,* 1977).

The prolongation of storage time at 5ºC increased significantly (P<0.01) amounts of AST and ALT enzymes released into the extracellular medium either the centrifuged or non- centrifuged semen.

Fertility rate and sex ratio:

The obtained results in Table 7 showed that the mean fertility rates were 70.59% in rabbit does artificially inseminated with the non- centrifuged semen and 74.19, 77.78, 80.56, 76.32 and 71.33% in centrifuged semen at 250, 500, 750, 1000 and 1250 *xg* for 15 minutes, respectively. The differences in mean fertility rates of the rabbit does artificially inseminated with the centrifuged semen were insignificantly higher than with non centrifuged semen. Similarly, no differences were recorded between the centrifuged semen at the different forces although the highest value of the fertility rate was recorded with the does artificially inseminated with the centrifuged semen at 750 *xg* for 15 minutes which may be due to the increase the percentage of sperm motility. Aitken *et al.* (1983) found a close correlation between spermatozoa movement of human semen and their penetrating ability into cervical mucus. In addition, Alexander (1981) and Murase *et al.* (1990) reported that the duration of sperm motility and penetration distance in the mucus were closely correlated to the pregnancy and conception rate.

Centrifugation of semen at different forces (250, 500, 750, 1000 and 1250 *xg* for 15 minutes) through Percoll gradients before artificial

Table 7. Kindling rate total number of kits and sex ratio of the doe rabbits artificially inseminated with the non-centrifuged and centrifuged spermatozoa.

Items	Non-centrifuged spermatozoa	Centrifuged spermatozoa (*xg*/15 minutes)				
		250	500	750	1000	1250
No. of does inseminated	34	31	27	36	38	42
No. of does conceived	24	23	21	29	29	30
Kindling rate	70.59	74.19	77.78	80.56	76.32	71.33
Total number of kits	7.2 ±1.2	7.9 ±1.1	7.5 ±1.4	8.4 ±0.9	8.1 ±1.2	7.4 ±0.8
Sex ratio :						
Females (%)	51.38 [c] ±1.25	53.16 [c] ±2.31	57.33 [b] ±1.12	60.71 [ab] ±1.65	62.96 [a] ±1.30	52.70 [c] ±2.15
Males (%)	48.61 [a] ±1.48	46.84 [a] ±1.82	42.67 [b] ±1.76	39.29 [bc] ±1.58	37.04 [c] ±1.6	47.30 [a] ±1.28

a-c: Means with different superscripts in the some row, differ significantly (P<0.05).

insemination increased significantly (P<0.05) the proportion of female kits by 1.78, 5.95, 9.33, 11.58 and 1.32 %, while decreased significantly (P<0.05) the proportion of the male kits by 1.77, 5.94, 9.32, 11.57 and 1.31% compared to the non- centrifuged semen. However, total number of kits at birth was not significant. The small increase of female kits, may reflect an enrichment of X-bearing spermatozoa in the 90% Percoll layer. Again, the results were not conclusive and need further investigations using a higher number of does. However, this small deviation in the proportion of females kits agreed with the degree of enrichment of X – bearing sperm reported in previous studies after reevaluation by fluorescence *in situ* hybridization : 52.5-55.1% in human spermatozoa (Wang *et al.*, 1994), 52.5-55.7% in bovine spermatozoa (Kobayashi *et al.* , 2004) and 51.0 – 54.8% in rabbit (Vega *et al.*, 2007).

In conclusion, centrifugation of rabbit semen at either 500 or 750 *xg* for 15 minutes through Percoll gradients had a beneficial effect on the viability of spermatozoa and kindling rate and produced a small increase in the proportion of female kits. This method could be valuable in mother – producing rabbit – breeding farms, owing to its simplicity and convenience.

REFERENCES

Aitken, R.J., Best, F., Richardson, D.W., Schats, R. and Simm, G. (1983). Influence of caffeine on movement characteristics, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. *Journal of Reproduction and Fertlity*, **67** : 19-27.

Alexander, N.J. (1981). Evaluation of male infertility with an in vitro cervical mucus penetration test. *Fertil. Steril.*, **38** : 201-208.

Boussit, D. (1989). Reproduction et insemination artificielle en cuniculure. Edite par I Association Francaise de Cuniculture. Lempdes, France.

Check, J.H. and Katsoff, D. (1993). A prospective study to evaluate the efficiency of modified swim–up preparation for male sex selection. *Hum. Reprod*., **8** , 211-214.

Corteel, J. (1975). Effect of washing on deep frozen goat semen preservation. *Ann. Biol. Anim. Biochem. Biophys*, **15** : 525-528.

Duncan, D.B. (1955). Multiple range and multiple F-test. *Biometrics*, **11**: 1-42.

El-Kelawy, H.M. (2002). Effect of centrifugation and c-AMP stimulators on motility , acrosome status and some enzymatic release of cooled rabbit spermatozoa. *Proceeding 3rd Science Conference On Rabbit Production In Hot Climates*, Hurghada, Egypt, pp. 263-274.

Ericsson, R.J., Langevin, C.N. and Nishino, M. (1973). Isolation of fractions rich in human Y sperm. *Nature*, **246** : 421-424.

Evans, G. and Maxwell, W.C.M. (1987). Salamon's Artificial Insemination of sheep and Goats. *Butterworth, Sydney* (Australia) pp. 194.

Garner, D.L., Gledhill, B.L., Pinkel, D. Lake, S., Stephenson, D., Van Dilla, M.A. and Johnson, L.A. (1983). Quantification of the X- and Y- chromosome bearing spermatozoa of domestic animals by flow cytometry. *Biol. Reprod.*, **28** : 312-321.

Goyal, R.L., Tuli, R.K., Georgie, G.C. and Chand, D. (1996). Comparison of quality and freezability of water buffalo semen after washing or sephadex filtration. *Theriogenology*, **46** : 679-686.

Jasko, D.J., Moran, D.M., Farlin, M.E. and Squires, E.L. (1991). Effect of seminal plasma dilution on or removal on spermatozoa motion characteristics of cooled stallion semen. *Theriogenology* , **35** : 1059-1062.

Kobayashi, J., Oguro, H., Uchida, H., Kohsaka, T., Sasada, H., Sato, E. (2004). Assessment of bovine X- and Y- bearing spermatozoa in fractions by discontinuous Percoll gradients with rapid fluorescence in situ hybridization. *Journal of Reproduction. Dev*., **50** : 463-469.

Lizuka, R., Kaneko, S., Aoki, R. and Kobayashi, T. (1987). Sexing of human sperm by discontinuous Percoll density gradient and its clinical application. *Hum. Reprod.*, **2** : 573-575.

Martinus, R.D.; Molar, P.C. and Shannon, P. (1991). Deleterious effects of seminal plasma in the cryopreservation of bovine spermatozoa. *N.Z.J. Res*. **34**: 281-286.

Memon, M.A., Bretzlaff, K.N. and Outt, R.S. (1985). Effect of washing on motility and acrosome morphology of frozen –thawed thawed goat spermatozoa. *American Journal of Vet. Research*, **46**: 473:475.

Murase, T., Okuda, K. and Sato, K. (1990). Assessment of bull fertility using mucus penetration test and human chorionic gonadotropin stimulation test. *Theriogenology*, **34** : 801-812.

Ng, F.L.H., Liu, D.Y. and Bakr, H.W.G. (1992). Comparison of Percoll, mini- Percoll and swin- up methods for sperm preparation from abnormal semen samples. *Human Reproduction*, **7** : 261-266.

Parrish, J.J., Krogenaes, A. and Susko-Parrish, J.L. (1995). Effect of bovine sperm separation by either swim- up of Percoll method on successs of in vitro fertilization and early embryonic development. *Journal of Theroogenology*, **43**: 859-869.

Quintela, I.A., Pena, A.I., Vega, M.D., Gullon, J., Prieto, C., Barrio, M., Becerra, J.J., Maseda, F. and Herradon, P. G. (2004). Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. *Reprod. Nutr. Dev*., **44** : 79-88.

Reitman, S. and Frankel, S. (1957). A colorimetric methods for the determination of serum glutamic oxaloacctic transminases. *American Journal of Clin. and Path*., **2**: 56-61.

Roca, J., Carrizosa J.A., Campos, I., Lafuente, A., Vazquez, J.M. and Martinez, E. (1997). Viability and fertility of unwashed Murciano-Grandina goat spermatozoa diluted in Tris- egg yolk extender and stored at 5ºC. *Small Ruminant Research*. **25**: 147-153.

Rombe, S., Kotjagina, V. and Piler, N. (1965). An improved method of preserving semen at -79 ºC. *Konevod Konnyi Sport*, **35** : 34 (Animal Breeding Abstract, 33, No. 2014).

Roy, A. (1957). Egg yolk coagulating enzymes in the semen and cowper's gland of the goat. *Nature* , **179** : 318-319.

Sanchez, R. Risopatron, J. Sepulveda, G. Pena, L. and Miska, W. (1995). Evaluation of the acrosomal membrane in bovine spermatozoa : Effect of protinase inhibitors. *Theriogenology*, **43** : 761-768.

Schill, W.B. and Wolff, H.H. (1974). Ultrastructure of human sperm acrosome and determination of acrosome activity under conditions of semen preservation. *International Journal of Fertility*, **19** : 217-223.

Sengupta, B.P., Singh, I.N. and Rawal, J. S. (1977). Areversible spermiostatic factor present in buffalo seminal plasma. *Cutt. Science*, **45** : 258-260.

Snedecor, G. W. and Cochran, W.G. (1982). *Statistical Methods*. 7th Edition, Iowa State University, Press Ames, USA .

Steeno, O., Adimoelja, A. and Steeno, J. (1975). Separation of X – and Y-bearing human spermatozoa with the Sephadex gel- filtration method. *Andrologia*, **7** : 95-97.

Vega, M.D., Pena, A.I.; Gullon, J., Prieto, C., Barrio, C., Becerra, J.J., Herradon, P.G. and Quintela, L.A. (2007). Sex ratio in rabbit following modified artificial insemination. *Animal Reproduction* Science, **5**: 1-7.

Wang, H.X., Flaherly, S.P., Swann, N.J. and Matthews, C.D. (1994). Discontinuous Percoll gradients enrich X – bearing human spermatozoa a study using double table fluorescence in situ hybridization. *Hum. Reproduction,* **9** : 1265-1270.

Watson, P.F. (1975). Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Record.*, **97** : 12-15.

Watson, P.F., Kutze, E., Cramer, P. and Hammerstedt, R.H. (1992). A comparison of critical osmolality and hydroaulic conductivity and its activation energy in fowl and bull spermatozoa. *Journal of Andrologia,* **13** : 131-138.

Zeidan, A.E.B., Abd El-Kariem, M.A., Abu El-Ela, A.A. and Ahmadi, E.A. (2004). Viability, enzymatic activities and penetrating ability of the washed goat spermatozoa into cervical mucus added with caffeine. *Proceeding 3rd International Conference on Animal, Poultry and Fish Production And Health in Semi – Arid Areas,* El-Arish , North Sinai, Egypt, pp. 160-171.

نوعية السائل المنوى ، معدل الإخصاب والنسبة الجنسية للحيوانات المنوية للأرانب بعد الطرد المركزى

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استخدم فى هذه الدراسة عدد عشرون ذكر ومائتين وثمانية أنثى أرنب نيوزيلندى أبيض (NZW). تم جمع وتقسيم السائل المنوى إلى ستة أجزاء متساوية. ترك الجزء الأول بدون طرد مركزى (مجموعة المقارنة) وتم معاملة الجزء الثانى والثالث والرابع والخامس والسادس بالطرد المركزى مع محلول البيركول على 250 ، 500، 750 ، 1000 و 1250 لفه لمدة 15 دقيقة على التوالى ثم بعد ذلك تم تخفيف كتلة السائل المنوى المترسبة بعد الطرد المركزى لتصل إلى تركيز 60 × 10 ^{6} حيوان منوى/ مل بمخفف السكروز – سترات. بعد ذلك حفظ السائل المنوى المخفف سواء بعد الطرد المركزى أو بدون الطرد المركزى على درجة حرارة 5° م لمدة صفر ، 1، 2 و 3 أيام ، ثم تم تقدير نوعية السائل المنوى ، النشاط الإنزيمى ، كذلك تم قياس نسبة الإخصاب والنسبة الجنسية لإناث الأرانب الملقحة اصطناعياً سواء بالسائل المنوى بعد الطرد المركزى أو بدون الطرد المركزى.

أوضحت النتائج أن هناك زيادة فى النسبة المئوية لحيوية الحيوانات المنوية معنوياً (على مستوى 0.01) ، مع انخفاض النسبة المئوية للحيوانات المنوية الميتة والشاذة وشواذ الأكروسوم ومعدل إرتشاح إنزيمات الاسبرتات – أمينوترانسفيريز (AST) والألانين – أمينو ترانسفيريز (ALT) إلى

البيئة الخارجية وذلك فى الحيوانات المنوية بعد الطرد المركزى مقارنة بالحيوانات المنوية بدون الطرد المركزى. كان أقصى نسبة لحيوية الحيوانات المنوية بدرجة معنوية (على مستوى 0.01) وأقل نسبة للحيوانات المنوية الميتة والشاذة وشواذ الأكروسوم وكمية إنزيمات AST و ALT سجلت عند طرد السائل المنوى على 500 أو 750 لفة لمدة 15 دقيقة. انخفاض النسبة المئوية لحيوية الحيوانات المنوية معنوياً (على مستوى 0.01) مع زيادة النسبة المئوية للحيوانات المنوية الميتة والشاذة وشواذ الأكروسوم وكمية إنزيمات AST و ALT معنوياً (على مستوى 0.01) مع تقدم فترة الحفظ على درجة حرارة 5° م لمدة ثلاثة أيام سواء فى الحيوانات المنوية المطرودة أو الغير مطرودة مركزياً. لم تختلف معنوياً نسبة الإخصاب وعدد الخلفات عند الميلاد للإناث الملقحة اصطناعياً بالسائل المنوى بعد الطرد المركزى أو بدون طرد مركزى على الرغم من زيادة معدل الإخصاب بدرجة غير معنوية عند إجراء الطرد المركزى للسائل المنوى على 500 أو 750 لفة لمدة 15 دقيقة مقارنة بالسائل المنوى بعد الطرد المركزى على 250 ، 1000 و 1250 لفة لمدة 15 دقيقة ، فى حين أن تلقيح الإناث بالسائل المنوى بعد الطرد المركزى أدى إلى زيادة عدد الخلفات الإناث معنوياً (على مستوى 0.05) مع انخفاض عدد الخلفات الذكور معنوياً (على مستوى 0.05) مقارنة بالسائل المنوى بدون الطرد المركزى.