American-Eurasian J. Agric. & Environ. Sci., 4 (3): 318-325, 2008
ISSN 1818-6769

Viability, Acrosomal Status and Sex Ratio of the Centrifuged Rabbit Spermatozoa

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Abstract: Twenty bucks and two-hundred eight multiparous lactating New-Zealand White (NZW) rabbit does, were used in the present study. Semen was collected, pooled 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 re-suspended to a final concentration of 60 x 10^6 sperm/ml in sucrose-yolk-citrate extender. The extended semen samples were then stored at 5°C for 3 days. After each storage period (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 assessed also.The obtained results showed that the percentage of motile spermatozoa and live spermatozoa with intact acrosome increased significantly ($P<0.01$), while the percentages of the live detached acrosome and dead spermatozoa with intact or detached acrosomes 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 spermatozoa with the live intact acrosome and the lowest ($P<0.01$) values of the live detached and dead spermatozoa with intact or detached acrosomes 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 and spermatozoa with the live intact or detached acrosomes decreased significantly ($P<0.01$), while the percentages of dead spermatozoa with intact or detached acrosomes and amounts of AST and ALT enzymes were increased significantly ($P<0.01$) with the increase of storage periods at 5°C 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 female kits and decreased significantly ($P<0.05$) the proportion of the male kits as compared to the non-centrifuged semen.

Key words: Rabbit semen · Centrifugation · Percoll gradient · Fertility · Sex ratio

INTRODUCTION

Spermatozoa interact with the surrounding medium which modulates sperm response to various stimuli. Reproductive performance attainable with artificial insemination also depends on the characteristics and/or the duration of exposure to various natural or artificial fluids such as epididymal secretion, seminal plasma, dilution media and oviductal fluids.

Rabbit semen quality and fertility can be improved by separating live spermatozoa from the ejaculate. Various techniques have been developed to harvest a homogeneous population of normal spermatozoa.

On the other hand, the role of seminal plasma on mature spermatozoa has been widely studied with contrasting results. Seminal plasma supports human, bull and ram sperm motility [1] by improving the viability and membrane integrity of the cells [2]. However, controversial results have been obtained and it is difficult to establish the effects of seminal plasma since, complex interactions are involved [3,4].

Since the 1970,s several investigators have made attempts to separate X and Y bearing spermatozoa by means of various gradient techniques, such as discontinuous albumin gradients [5], sephadex columns [6], discontinuous Percoll gradients [7] or the swim-up

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procedure [8]. 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 [9]. 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 [10].

The effect of seminal plasma in rabbit semen is not completely known. Therefore, 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, Egypt.

Twenty bucks and two hundred eight multiparous lactating New Zealand White (NZW) rabbit does 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.* [11].

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 portions. In the first portion, semen was non-centrifuged (control). The second, third, fourth, fifth and sixth portions were centrifuged (treated) at 250, 500, 750, 1000 and 1250 *xg* for 15minutes through Percoll gradient according to Vega *et al.* [12]. 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 put 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 [13]. 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 5°C over 2 h and then stored at this temperature for up to 3 days. After each storage period (0, 1, 2 and 3 days), percentages of sperm motility and acrosomal status (a. live intact acrosome, b. live detached acrosome, c. dead intact acrosome and dead detached acrosome) were recorded according to Didion *et al.* [14]. 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 Reitman and Frankle [15].

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 [16]. Rabbit does were palpated to establish pregnancy after 14 days of insemination. The fertility rates of the rabbit does 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 [17]. The significant differences among means were evaluated by Duncans New Multiple Range test [18]. 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

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

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$67.25^{a}\pm2.01$	$69.32^{a}\pm1.76$	$71.92^{a}\pm1.45$	$73.12^{a}\pm1.65$	$71.18^{a}\pm2.28$	$71.13^{a}\pm2.13$
1	$50.22^{b}\pm1.82$	$61.42^{b}\pm2.38$	$67.18^{b}\pm1.82$	$67.18^{b}\pm1.28$	$66.24^{b}\pm1.16$	$64.13^{b}\pm1.35$
2	$39.52^{c}\pm2.10$	$52.63^{c}\pm1.46$	$55.16^{c}\pm1.90$	$58.25^{c}\pm2.13$	$53.42^{c}\pm2.08$	$52.54^{c}\pm1.72$
3	$26.14^{d}\pm1.42$	$34.16^{d}\pm1.28$	$40.35^{d}\pm1.17$	$42.36^{d}\pm1.14$	$40.38^{d}\pm1.16$	$35.19^{d}\pm1.18$
Means±SE	$45.78^{E}\pm1.72$	$54.64^{D}\pm1.48$	$58.65^{AB}\pm1.39$	$60.23^{A}\pm1.32$	$57.81^{BC}\pm1.37$	$55.76^{C}\pm1.55$

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)

Table 2: Mean percentage of the live intact acrosome of the non-centrifuged and centrifuged rabbit spermatozoa, during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$60.32^{a}\pm1.85$	$64.51^{a}\pm1.72$	$70.48^{a}\pm1.89$	$71.64^{a}\pm1.92$	$70.52^{a}\pm2.13$	$67.42^{a}\pm1.95$
1	$54.75^{b}\pm1.18$	$58.22^{b}\pm2.13$	$64.16^{b}\pm2.18$	$66.53^{b}\pm1.28$	$63.81^{b}\pm1.91$	$58.64^{b}\pm1.72$
2	$46.28^{c}\pm1.26$	$51.35^{c}\pm2.05$	$57.24^{c}\pm1.52$	$60.41^{c}\pm1.16$	$55.86^{c}\pm1.88$	$53.85^{c}\pm1.35$
3	$37.16^{d}\pm1.72$	$43.28^{d}\pm1.19$	$51.20^{d}\pm1.38$	$53.15^{d}\pm1.28$	$50.91^{d}\pm1.28$	$46.75^{d}\pm1.17$
Means	$49.63^{C}\pm1.52$	$54.34^{B}\pm1.87$	$60.77^{A}\pm1.18$	$63.06^{A}\pm1.73$	$60.28^{A}\pm1.86$	$56.67^{B}\pm1.83$

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

A-C: Means with different superscripts in the same row, differ significantly (P<0.01)

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.* [19] found that centrifugation of bovine 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 [20]. Corteel [21] found that low molecular weight component was retained with spermatozoa and the high molecular weight fraction was removed by centrifugation. Jasko *et al.* [22] also confirmed that high molecular weight fractions in seminal plasma depress sperm motility, livability and absolute index of livability. Bahgat [23] showed that centrifugation of rabbit semen on Percoll gradient at 500 or 750 *xg* for 15 minutes significantly increased sperm motility. Zeidan *et al.* [24] also found that the highest value of sperm motility was recorded in the centrifuged semen at 950 *xg* for 15 minutes in goats semen. Viability of spermatozoa was reduced with increasing centrifugation time 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 [25]. This damage may increase during centrifugation and washing which are required for sperm selection [26].

Data presented in Table 2, 3, 4 and 5 showed that the effects of type of rabbit spermatozoa (centrifuged or non-centrifuged) on percentages of the live or dead spermatozoa with intact or detached acrosomes were highly significant (P<0.01). The percentages of the live spermatozoa with intact acrosome increased significantly (P<0.01), while the percentages of the dead spermatozoa with intact or detached acrosomes were decreased significantly (P<0.01) after centrifugation at different forces (250, 500, 750, 1000 and 1250 *xg* for 15 min) as compared to the non-centrifuged spermatozoa. The percentages of the live spermatozoa with intact acrosome were significantly (P<0.01) higher after centrifugation at 500, 750 and 1000 as compared to 250 and 1250 *xg* for 15 minutes or the non-centrifuged spermatozoa. The lowest (P<0.01) value of the percentages of the live spermatozoa with the intact acrosomes was recorded with the non-centrifuged spermatozoa, while the highest (P<0.01) values of the percentages of the live intact acrosome were recorded with the centrifuged spermatozoa

Table 3: Mean percentage of the live detached acrosome of the non-centrifuged and centrifuged rabbit spermatozoa, during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$4.52^{b}\pm0.45$	$4.15^{b}\pm0.28$	$2.65^{b}\pm0.26$	$2.48^{b}\pm0.35$	$2.81^{b}\pm0.33$	$4.07^{b}\pm0.26$
1	$5.78^{a}\pm0.38$	$5.61^{a}\pm0.31$	$3.12^{a}\pm0.28$	$2.95^{a}\pm0.19$	$3.42^{a}\pm0.24$	$5.42^{a}\pm0.21$
2	$4.26^{b}\pm0.58$	$3.87^{b}\pm0.18$	$2.74^{b}\pm0.17$	$2.26^{b}\pm0.22$	$2.85^{b}\pm0.28$	$3.85^{b}\pm0.25$
3	$3.88^{c}\pm0.16$	$3.32^{c}\pm0.28$	$2.11^{c}\pm0.16$	$1.86^{c}\pm0.12$	$2.16^{c}\pm0.12$	$3.15^{c}\pm0.17$
Means	$4.61^{A}\pm0.41$	$4.24^{A}\pm0.49$	$2.66^{B}\pm0.21$	$2.39^{B}\pm0.23$	$2.81^{B}\pm0.26$	$4.12^{A}\pm0.47$

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

A-B: Means with different superscripts in the same row, differ significantly (P<0.01)

Table 4: Mean percentage of the dead intact acrosome of the non-centrifuged and centrifuged rabbit spermatozoa, during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$31.50^{d}\pm0.85$	$25.75^{d}\pm0.63$	$24.11^{d}\pm0.42$	$20.84^{d}\pm1.02$	$24.08^{d}\pm0.88$	$26.21^{d}\pm0.82$
1	$34.81^{c}\pm0.65$	$28.16^{c}\pm0.85$	$26.15^{c}\pm0.79$	$24.68^{c}\pm0.88$	$28.17^{c}\pm1.10$	$30.13^{c}\pm0.78$
2	$39.65^{b}\pm0.83$	$32.82^{b}\pm0.78$	$30.12^{b}\pm0.86$	$29.81^{b}\pm0.91$	$31.10^{b}\pm0.75$	$34.12^{b}\pm0.95$
3	$46.23^{a}\pm1.02$	$38.66^{a}\pm1.12$	$35.22^{a}\pm0.92$	$32.90^{a}\pm0.79$	$36.13^{a}\pm0.68$	$41.18^{a}\pm1.12$
Means	$38.05^{A}\pm0.63$	$31.35^{B}\pm0.56$	$28.93^{D}\pm0.48$	$27.06^{B}\pm0.53$	$29.87^{C}\pm0.50$	$32.91^{B}\pm0.63$

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: Mean percentage of the dead detached acrosome of the non-centrifuged and centrifuged rabbit spermatozoa, during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$5.01^{d}\pm0.18$	$3.29^{d}\pm0.43$	$3.20^{d}\pm0.28$	$3.11^{d}\pm0.18$	$3.42^{d}\pm0.56$	$3.25^{d}\pm0.41$
1	$6.11^{c}\pm0.23$	$4.66^{c}\pm0.18$	$4.18^{c}\pm0.61$	$3.87^{c}\pm0.38$	$4.15^{c}\pm0.62$	$4.58^{c}\pm0.62$
2	$8.15^{b}\pm0.53$	$6.28^{b}\pm0.71$	$5.14^{b}\pm0.18$	$4.75^{b}\pm0.56$	$6.05^{b}\pm0.58$	$5.91^{b}\pm0.38$
3	$12.17^{a}\pm0.48$	$9.42^{a}\pm0.28$	$8.12^{a}\pm0.35$	$7.56^{a}\pm0.81$	$8.27^{a}\pm0.64$	$9.38^{a}\pm0.27$
Means	$7.86^{A}\pm0.31$	$5.91^{C}\pm0.26$	$5.16^{CD}\pm0.21$	$4.82^{D}\pm0.19$	$5.47^{BC}\pm0.21$	$5.78^{B}\pm0.26$

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)

at 750 *xg* for 15 minutes and the percentages of the live detached acrosome and dead intact or detached acrosomes of spermatozoa were recorded with the non-centrifuged spermatozoa. These findings may be attributed to centrifugation and the toxic components through the drainage of seminal plasma which cause the acrosomal damage. Jones and Stewart [27] indicated that extension and cooling of the bull semen to 5°C caused acrosomal swelling in about 50% of the spermatozoa. In addition, the intact acrosomes carry hydrolytic enzymes necessary for oocytes penetration, therefore, have a strong relationship with the fertility [28]. Martinus *et al.* [29] reported that the presence of seminal plasma caused an increase in the 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 [30, 31]. 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 [32].

The prolongation of storage time at 5°C decreased significantly (P<0.01) the percentage of sperm motility and spermatozoa with the live intact or detached acrosomes,

Table 6: Aspartate aminotransferase (U/10^6 spermatozoa) enzyme activity of the non-centrifuged and centrifuged rabbit semen during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$61.18^{d}\pm2.11$	$42.19^{d}\pm1.17$	$40.65^{d}\pm1.52$	$35.48^{d}\pm1.25$	$40.16^{d}\pm1.12$	$52.12^{d}\pm1.25$
1	$68.22^{c}\pm1.82$	$48.11^{c}\pm1.38$	$43.50^{c}\pm1.38$	$39.72^{c}\pm1.18$	$43.20^{c}\pm2.13$	$58.13^{c}\pm1.31$
2	$82.44^{b}\pm1.65$	$56.13^{b}\pm2.15$	$50.38^{b}\pm2.14$	$46.52^{b}\pm1.67$	$54.35^{b}\pm1.17$	$65.18^{b}\pm1.52$
3	$90.16^{a}\pm2.13$	$67.18^{a}\pm2.16$	$58.25^{a}\pm1.82$	$54.81^{a}\pm1.72$	$61.18^{a}\pm1.22$	$74.14^{a}\pm2.18$
Means	$75.49^{A}\pm1.30$	$53.40^{C}\pm1.07$	$48.19^{D}\pm0.78$	$44.38^{E}\pm0.80$	$49.72^{D}\pm0.97$	$62.39^{B}\pm0.94$

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)

Table 7: Alanine-aminotransferase (U/10^6 spermatozoa) enzyme activity of the non-centrifuged and centrifuged rabbit semen during storage at 5°C for up to 3 days

		Centrifuged spermatozoa (*xg* for15 minutes)				
Storage period (Days)	Non-centrifuged spermatozoa	250	500	750	1000	1250
0	$12.41^{d}\pm0.53$	$7.25^{d}\pm0.72$	$5.25^{d}\pm0.32$	$5.17^{d}\pm0.28$	$6.16^{d}\pm0.31$	$10.72^{d}\pm0.71$
1	$15.72^{c}\pm0.81$	$9.72^{c}\pm0.42$	$7.82^{c}\pm0.48$	$6.35^{c}\pm0.66$	$9.22^{c}\pm0.82$	$13.52^{c}\pm0.48$
2	$19.82^{b}\pm0.72$	$13.85^{b}\pm0.38$	$11.13^{b}\pm0.82$	$8.19^{b}\pm0.42$	$12.51^{b}\pm0.55$	$16.48^{b}\pm1.02$
3	$25.31^{a}\pm1.02$	$20.16^{a}\pm0.52$	$16.28^{a}\pm0.57$	$13.46^{a}\pm0.72$	$16.38^{a}\pm0.73$	$24.19^{a}\pm1.11$
Means	$18.32^{A}\pm0.50$	$12.75^{C}\pm0.56$	$10.12^{D}\pm0.47$	$8.29^{E}\pm0.36$	$11.07^{D}\pm0.43$	$16.23^{B}\pm0.57$

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)

while percentages of dead spermatozoa with the intact or detached acrosomes of the centrifuged rabbit spermatozoa at different forces or non-centrifuged spermatozoa were increased significantly (P<0.01). Similar trends were reported by Memon *et al.* [30], Roca *et al.* [31] and Zeidan *et al.* [24]. Ijaz and Hunter [33] demonstrated that more sperm without intact acrosomes were found after 4 h of storage than at 0 h, but the percentage did not change further until after 24 h of storage at 5°C.

Enzymatic Activities: As shown in Table 6 and 7, 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 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 750 *xg* for 15 minutes. These results were in agreement with those of Zeidan *et al.* [24] in goat and Bahgat [23] in rabbit spermatozoa. These results may be due to the removal of the inhibitory factors found in seminal plasma which caused cell membrane damage and increased AST and ALT release [34].

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

Fertility Rate and Sex Ratio: The obtained results in Table 8 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 in the percentage of sperm motility. Aitken *et al.* [35] found a close correlation between spermatozoa movement of

Table 8: Kindling rate, total number of kits and sex ratio of the doe rabbits artificially inseminated with the non-centrifuged and centrifuged spermatozoa

		Centrifuged spermatozoa (*xg* for15 minutes)				
Items	Non-centrifuged spermatozoa	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 (%)	51.39^{a}±1.25	53.16^{c}±2.31	57.33^{b}±1.12	60.71^{ab}±1.65	62.96^{a}±1.30	52.70^{a}±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)

human semen and their penetrating ability into cervical mucus. In addition, Alexander [36] and Murase *et al.* [37] 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 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%, respectively as compared to the non-centrifuged semen. However, total number of kits at birth was not significantly different. The little 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 little deviation in the proportion of female 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 [38], 52.5-55.7% in bovine spermatozoa [10] and 51.0-54.8% in rabbit [12].

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

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