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# EFFECT OF EXTENDER TYPE ON FREEZABILITY OF RAM SEMEN

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***ABSTRACT:*** ***Semen was collected from 4 adult Ossimi rams (2-4 years) by artificial vagina. Ejaculates having ≥80% motility and sperm concentration of ≥3 x $10^9$/ml were pooled together and gently mixed to study the effect of selected five extenders on freezability of ram spermatozoa by split –sample technique. Two extenders (A and B) were free of skim milk and three extenders (C, D and E) contained skim milk at various levels (35, 50 and 65%, respectively). The final glycerol level was 14.5% and the dilution rate was 1:6 (v/v). Semen was equilibrated for 3 h at 4-5 °C. The frozen semen was thawed in water bath at 42 °C for 35 seconds. Sperm motility, livability and abnormality were evaluated just before and after glycerolation, after equilibration and post–thawing of frozen semen.***

***Results indicated that extenders A and B have yielded significantly (P<0.01) the highest percentage of post–thawing motility (35.6 and 42.5%), livability (37.0 and 42.1%), and the least sperm abnormality (23.8 and 19.7%), as compared to 31.3, 23.1 and 21.3% for motility, 31.9, 25.4 and 23.3% for livability and 21.51, 24.99 and 26.74% for abnormality of extenders C,D and E, respectively. Post–thawing motility and viability progressively decreased as the skim milk content in the extender increased, while the post–thawing abnormality showed an opposite trend. The differences were significant (P<0.01). B-extender maintained superior post–thawing recovery rate of ram spermatozoa, being 58.6% for motility and 58.2% for livability relative to equilibrated values and 46.5 for motility and 46.1% for livability relative to initial values. Total abnormality increment was 17.68%. The least respective values for motility and viability and the higher value for abnormality were expressed by the E-extender, which contained the greatest skim milk. The loss in motility between equilibration and thawing ranged from 28.8 to 36.9% and the least values (5.6–8.7%) were obtained between glycerolation and equilibration. The corresponding values were 28.5–33.4% and 5.5–9.5% for livability and 12.37–15.72 % and 1.68–2.78% for abnormality, respectively. In conclusion, tris-based extenders have higher freezability for ram semen as compared to skim milk extenders.***

***Key words:*** ***Sheep, semen, freezing, motility.***

## INTRODUCTION

**Successful manipulation of artificial insemination through applying frozen semen is unfortunately still being beyond optimum. Improvement of such technology would accelerate the genetic improvement in sheep. One of the important factors influencing frozen storage of semen is the composition of the extender used for dilution of semen before freezing (Salamon and Maxwell, 2000). However, with adaptation of the early tested ram extenders, which were based on utilization of citrate buffer combined with monosaccharide (glucose), the results had some limitations and most have been modified later (Salamon and Maxwell, 1995). Milk is an isotonic medium containing many components favorable to the maintenance of sperm viability. Milk was used extensively by several investigators for liquid and frozen ram semen, mostly combined with fructose, sodium citrate or tris (Nebar, 1989; Söderquist *et al,* 1997; El–Alamy and Foote, 2001; Gil *et al.,* 2003 and Paulenz *et al.,* 2003). Moreover, Jones and Martin (1965) found that egg yolk extenders were inferior to skim milk extenders for freezing and *in vitro* incubation of ram spermatozoa after thawing. The efficiency of tris and milk extenders over egg yolk – citrate and egg yolk glucose to maintain ram spermatozoa was reported by Deka and Rao (1980). Similarity, Nebar (1989) found that tris–based extenders maintained, in general progressive motility and acrosome integrity of ram frozen spermatozoa over skim milk – based extenders. Gil *et al.* (2000) recommended the utilization of the milk–based extenders for freezing of ram spermatozoa with low concentration of egg yolk (5–10%) and addition of glycerol at 5°C on two–step dilution method and 2 h–equilibrated period. The present work aimed to study the effect of five different extenders on freezability of ram spermatozoa, based on the percentage of motility, livability and abnormality of spermatozoa at various stages of freezing and thawing processing.**

## MATERIALS AND METHODS

**Semen was collected twice weekly from 4 adult healthy Ossimi rams (2-4 years) by artificial vagina at the Experimental Farm, Department of Animal Production, Faculty of Agriculture Minufiya University. Semen was evaluated from the rams for two weeks prior to the initiation of the experiment. Rams were fed a balanced ration meeting the NRC requirements for adult rams (NRC, 1987).**

**Immediately after semen collection, every ejaculate was evaluated for color, motility and sperm concentration. Ejaculates having 80% or more progressive motility and sperm concentration of not less than 3x$10^9$/ml were pooled together and gently mixed. The pooled semen sample was split into 5 aliquots to study the effect of the selected five extenders on freezability of ram semen. The composition of these extenders is shown in Table 1.**

**Table 1: Composition of semen extenders.**

| Component | Tris–based extenders | | Skim milk–tris–based extenders | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Tris*, g | 2.422 | 2.422 | 1.574 | 1.211 | 0.848 |
| Citric acid, g | 1.340 | 1.340 | 0.871 | 0.670 | 0.469 |
| $Na_3$ – Citrate, g | - | 0.145 | 0.094 | 0.073 | 0.051 |
| Fructose, g | 1.000 | 0.750 | 0.111 | 0.085 | 0.060 |
| Egg yolk, ml | 20.0 | 20.0 | 13.0 | 10.0 | 7.0 |
| Skim milk, ml | - | - | 35 | 50 | 65 |

- * Tris: Hydroxymethyl aminomethane.

- Distilled water was added up for100 ml to extenders A, B, and which mixed with skim milk (C, D and E).

- Antibiotics were added as 75 mg penicillin plus 50 mg streptomycin

Each semen extender was divided into two equal fractions namely; fraction I being free of glycerol and fraction II which was glycerolated. Glycerol was added to fraction II at a level of 29 %. In the meantime, the pooled semen sample was divided into five equal aliquots, and each aliquot was partially diluted by adding three volumes of fraction I of one of the selected extenders, so that the preliminary dilution rate was 1: 3 (volume/volume). The temperature of both semen and fraction I was 30-32 °C at such partial dilution. Then the partially diluted semen was gradually cooled to 4-5 °C over 1.5 h. Glycerolation was carried out at 5 °C by adding an equal volume of the appropriate glycerolated extender (fraction II) to the unglycerolated fraction I of diluted semen in 3 parts over an interval of 15 minutes. The final glycerol concentration after adding fraction II was 14.5% and the final dilution rate was 1:6 (volume/volume). Thereafter, the glycerolated semen was equilibrated at 4 – 5 °C for 3 hours. After equilibration, the semen was packed in the 0.5 ml French plastic straws, which were sealed using polyvinyl powder. Three straws for each extender were prepared, labeled and coded. The cold straws were mounted on metal rack and frozen in ethyl alcohol to -16.5 °C over 4 minutes and the freezing rate is shown in Table (2). Thereafter, the straws were dipped in the liquid nitrogen (-196°C) and stored for 24 hours. The frozen semen was thawed in water bath at 42 °C for 35 seconds.

**Table 2: Preliminary freezing rate of the filled straws in ethyl alcohol and time spent to reach the subzero temperature –16.5 °C.**

| Temp., °C | zero | -4.0 | -0.7 | -0.9 | -10.5 | -12.0 | -13.5 | -15.0 | -16.5 |
|---|---|---|---|---|---|---|---|---|---|
| Time, min | 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |

**The percentages of sperm motility, livability and abnormality were determined at the following stages during semen processing:**

1. **Fresh semen: after pooling satisfactory ram ejaculates and gently mixed (Initial motility, livability or abnormality).**
2. **After glycerolation (G).**
3. **After equilibration (E).**
4. **Post thawing (PT).**

**Reduction in the percentage of sperm motility and livability during the various stages was calculated as follow:**

**Stage 1: The difference between the % of initial motility or livability and % of the motility or livability after glycerolation = (I-G).**

**Stage 2: The difference between the % of motility or livability after glycerolation and % of the motility or livability after equilibration = (G-E).**

**Stage 3: The difference between the % of motility or livability after equilibration and the % of motility or livability post thawing = (E-PT).**

**Total reduction: the difference between the % of initial motility or livability and the % of motility or livability post thawing = (I-PT).**

**All the processing steps and the previous evaluations were replicated eight times. The obtained results were statistically analyzed according to Snedecor and Cochran (1980).**

## RESULTS AND DISCUSSION

**Data listed in Table (3) indicate that the type of extender significantly (P<0.01) affected the percentage of sperm motility and livability. In this respect, the tris–citric acid– fructose–egg yolk–glycerol-based extenders (B and A extenders) yielded significantly (P<0.05) higher post-thawing motility (42.5 and 35.6%, respectively) and livability (42.1 and 37.0, respectively) than that resulted after extension with skim milk–tris-citric acid–based extenders (C, D and E, being 31.3, 23.1 and 21.3% for motility and 31.9, 25.4 and 23.3%for livability, respectively; Table 3). The superiority of B-extender in maintaining post thawing motility (42.5%) and livability (42.1%) of spermatozoa than A extender (35.6 and 37.0%, respectively ) could be due to difference in its fructose content (1.0 g in A vs. 0.75 g in B) and sodium citrate existing only in B-extender but was not present in A extender (Table 1). It means that addition of sodium citrate beside the slight decrease in fructose level of the tris based egg yolk–glycerol extender seems to improve the post–thawing motility and livability. In agreement with the present differences among different types of extenders, Paulenz *et al.* (2002) found that ram spermatozoa diluted in tris based extender showed higher sperm motility and membrane integrity than those diluted in milk based extender. In the same time, Foote and Kaproth (2002) indicated that percentage of motile bull sperm significantly (P<0.01) increased in response to the addition of 1.0% fructose to whole milk–glycerol diluent. In nearly similarity with the present results, El-Maghraby (2007)**

**revealed that the post thawing sperm motility of goat semen diluted with tris-based extender was 42.3%.**

**Table (3): Effect of extender type on percentage of motility, livability and abnormality of ram spermatozoa at the various stages of freezing and thawing.**

| Stage of semen processing | Extender type | | | | |
|---|---|---|---|---|---|
| | Tris-based extender | | Tris-skim milk based extender | | |
| | A | B | C | D | E |
| **Sperm motility (%) :** | | | | | |
| Unglycerolated (UG) | 84.4 $^{a}$ | 85.0 $^{a}$ | 80.0 $^{b}$ | 72.5 $^{c}$ | 71.3 $^{c}$ |
| Glycerolated (G) | 73.1 $^{b}$ | 78.1 $^{a}$ | 71.9 $^{b}$ | 66.3 $^{c}$ | 64.4 $^{c}$ |
| Equilibration (E) | 64.4 $^{b}$ | 72.5 $^{a}$ | 65.0 $^{b}$ | 60.0 $^{c}$ | 56.0 $^{d}$ |
| Post thawing (PT) | 35.6 $^{b}$ | 42.5 $^{a}$ | 31.3 $^{c}$ | 23.1 $^{d}$ | 21.3 $^{d}$ |
| **Sperm livability (%) :** | | | | | |
| Unglycerolated (UG) | 85.8 $^{a}$ | 84.6 $^{a}$ | 78.9 $^{b}$ | 71.8 $^{c}$ | 69.4 $^{c}$ |
| Glycerolated (G) | 75.0 $^{ab}$ | 78.0 $^{a}$ | 71.2 $^{b}$ | 64.6 $^{c}$ | 61.3 $^{c}$ |
| Equilibration (E) | 65.5 $^{b}$ | 72.3 $^{a}$ | 64.9 $^{bc}$ | 58.8 $^{c}$ | 55.8 $^{c}$ |
| Post thawing (PT) | 37.0 $^{b}$ | 42.1 $^{a}$ | 31.9 $^{c}$ | 25.4 $^{d}$ | 23.3 $^{d}$ |
| **Sperm abnormality (%):** | | | | | |
| Unglycerolated (UG) | 2.85 $^{a}$ | 3.04 $^{a}$ | 3.07 $^{b}$ | 3.47 $^{c}$ | 3.5 $^{c}$ |
| Glycerolated (G) | 6.68 $^{b}$ | 5.48 $^{a}$ | 7.46 $^{b}$ | 8.01 $^{c}$ | 8.24 $^{c}$ |
| Equilibration (E) | 8.82 $^{b}$ | 7.22 $^{a}$ | 9.14 $^{b}$ | 9.66 $^{c}$ | 11.02 $^{d}$ |
| Post thawing (PT) | 23.83 $^{b}$ | 19.68 $^{a}$ | 21.51 $^{c}$ | 24.99 $^{d}$ | 26.74 $^{d}$ |

**Initial motility: 91.3 % Initial livability: 91.4 % Initial abnormality: 2.0 %**

**a, b, c, d: Values within the same row with different superscripts are significantly different (P<0.05).**

**It is worthy noting that, the post–thawing sperm motility and livability recorded for the extenders C, D and E progressively decreased as the skim milk increased and the citric acid, tris, sodium citrate, fructose and egg yolk decreased in the diluent (Table 1). Accordingly, it was not possible to isolate effect(s) of constituents of these diluents contents on such differences among these extenders. However, the differences in both egg yolk and skim milk content among such extenders are much more pronounced than that of the other constituents (Table 1). Thus the significant differences in percentage of post– thawing motility and livability detected in the present study among C, D, and E extenders may be attributed to differences in skim milk and/or egg yolk level rather than due to other constituents existed in such extenders.**

**Sansone *et al.* (2000) reported that majority of workers used egg yolk concentration around 20%. However, Gil *et al*. (2003) diluted ram semen with milk–based extenders containing four different levels of egg yolk (5, 10, 15, 20%), but they did not found significant positive effect of increasing the level of egg yolk above 10% on post–thawing motility. They added that the preparations containing 10% egg yolk gave significantly better post-thawing membrane integrity. In an earlier study, Sahni and Mohan (1990) found that egg yolk beyond 5% in tris–glycerol based freezing diluent did not show any significant improvement in respecting with the post-thaw sperm motility. However, their study clearly showed the scope of reduction of egg yolk level from 20% to 5% without any adverse effect on the freezability of buffalo semen. Kumar *et al.* (1992) reported that addition of egg yolk to the diluents stimulated an enzymatic system of the spermatozoa in addition to its protective action against cold shock. The confliction in respecting with the observed effects of addition of egg yolk at various levels on post–thaw sperm motility among the mentioned studies could be attributed to the interaction of the other components of the extender with the protective effect of egg yolk.**

**Akhtar *et al.* (1990) found that the tris–based extender was superior to milk diluent as determined by post–thawing motility, which agrees with the present study. An opposite trend was reported by Galli *et al.* (1993) who found that the milk–based extender was better than the tris–based extenders in respecting with the post–thawing sperm motility. In fact, the present study used the skim milk combined with tris–citrate buffer which may explain the confliction of the present finding and that of Galli *et al.* (1993).**

**Data in Tables (3 and 4) indicate that the sperm abnormality expressed an opposite trend to both motility and livability of ram spermatozoa with the advancement of processing stages. In other words, dilution, cooling, equilibration, freezing and thawing substantially increased the percentage of sperm abnormality. B–extender showed the least ($P<0.05$) percentage of sperm abnormality (Table 3). The skim milk-tris based extender (C-extender) maintained abnormality at significantly lower percentage than D- and E-extenders (Table 3). Farag *et al.* (1983) reported that the percentage of sperm coiled tail greatly increased in frozen ram semen and it was correlated with increasing of Zn influx.. A similar relationship was early achieved by Blom and Wolstrup (1976). Their finding may suggest differences in such effects depended on the extenders components.**

**Data in Table (4) indicate that the percentage of motile ram spermatozoa progressively decreased with the advancement of semen processing. In this respect, reduction in sperm motility was the greatest (28.8-36.9%) during stage 3 due to freezing and thawing, followed by that occurred during stage 1 (due to dilution and cooling), which varied from 13.2 to 26.9%. The least values of motility loss were recorded (5.6 to 8.7%) during equilibration (stage 2). Aisen**

***et al.*** **(2000) found that the percentage of sperm motility of ram was 78.8 after cooling and 58.3% after thawing, when they used tris–citric acid–fructose–egg yolk glycerol extender. This finding is almost similar with that obtained in the present study at cooling and glycerolated but it was greater at post–thaw than that obtained in the present study. Such differences in the results may be due to differences in the extender components, glycerol level or the freezing methods.**

**Table (4): Reduction of motility and livability and Increment of abnormality of ram spermatozoa during freezing process.**

| Extender | Stages of reduction | | | | Recovery rate (%) | |
|---|---|---|---|---|---|---|
| | 1 (I-G) | 2 (G-E) | 3 (E-PT) | Total | Initial | Equrli-bartion |
| **Reduction of motility:** | | | | | | |
| A | 18.20 | 8.70 | 28.80 | 55.70 | 39.0 | 55.3 |
| B | 13.20 | 5.60 | 30.00 | 48.80 | 46.5 | 58.6 |
| C | 19.40 | 6.90 | 33.70 | 60.00 | 34.3 | 48.2 |
| D | 25.00 | 6.30 | 36.90 | 68.20 | 25.3 | 38.5 |
| E | 26.90 | 8.40 | 34.70 | 70.00 | 23.3 | 38.0 |
| **Reduction of livability:** | | | | | | |
| A | 16.40 | 9.50 | 28.50 | 54.40 | 40.5 | 56.5 |
| B | 13.40 | 5.70 | 30.20 | 49.30 | 46.1 | 58.2 |
| C | 20.20 | 6.30 | 33.00 | 59.50 | 34.9 | 49.2 |
| D | 26.80 | 5.80 | 33.40 | 66.00 | 27.8 | 43.2 |
| E | 30.10 | 5.50 | 32.50 | 68.10 | 25.5 | 41.8 |
| **Increment of abnormality:** | | | | | | |
| | (G-I) | (E-G) | (PT-E) | Total | | |
| A | 4.68 | 2.14 | 15.01 | 21.83 | | |
| B | 3.48 | 1.74 | 12.46 | 17.68 | | |
| C | 5.46 | 1.68 | 12.37 | 19.51 | | |
| D | 6.01 | 1.65 | 15.33 | 22.99 | | |
| E | 6.24 | 2.78 | 15.72 | 24.74 | | |

**Initial motility: 91.3 %  Initial livability: 91.4 %  Initial abnormality: 2.0 %**

**Results further illustrate that the total depression of motility was the least for tris–citrate based extender either for B-extender (48.8%) or for A–exrender (55.7%) compared with skim milk–tris- citrate based extenders, being (60%, 68.2% and 70.0% for C, D and E extenders, respectively). It is of interest to note that the superiority of extenders A and B in maintaining sperm motility was associated with the least depression of motility during all freezing stages (Total reduction in sperm motility, So, it could be concluded that, tris–citrate based extender (free of skim milk) provided the spermatozoa with more suitable protection and environment against the effect of dilution, cooling, equilibration, freezing and thawing than that provided by skim milk–tris-citrate based extenders (C, D and E).**

**The post–thaw recovery rate of motility was the best for B-extender being 46.5% in the proportion to the initial motility in fresh semen and 58.6% in the proportional to the motility just after equilibration. The corresponding figures for C-extender were 34.3% and 48.2%, respectively (Table 4). At the same time, the percentage of sperm livability decreased considerably with the advancement of semen processing, but at variable rates. The greatest rate of depression of sperm livability occurred during freezing and thawing (from 28.5 to 33.4%), followed by that during dilution and cooling (13.4 to 30.1%) and the least depression action was due to equilibration (from 5.5 to 9.5%). Values of recovery rate of post–thawing viable ram spermatozoa are very close to the corresponding values of sperm motility (Tables 3 and 4), which suggest close association of both traits as reported by Abdel-Khalek *et al.* (2008).**

**Vishwanath and Shannon (2000) reported that even with the best preservation techniques and all the development occurred in semen processing, the best sperm recovery post thawing is just over 50%, which agrees with the present recovery rate in proportional to sperm motility in equilibratedsemen, but it is greater than the recovery rate in proportional to the initial motility. On the other hand, the post–thaw recovery rate of motility basis of data demonstrated by Aisen *et al.* (2000) was 74% in proportional to motility after cooling.**

**El–Alamy and Foote (2001) compared the effect of three different egg yolk ratios (i.e., 20, 30 and 40% v/v) in tris based ram extender on post–thaw motility, and they found it to be 41, 38 and 20% for Finn ram spermatozoa versus 47, 41 and 27% for Dorset ram spermatozoa, respectively. It means that the excess addition of egg yolk to tris– based extender at rates over 20% (v/v) adversely affected the post–thaw motility of ram spermatozoa. However, the recovery rate was 71, 65 and 36% for Finn ram semen versus 76, 66 and 43% for Dorset ram semen, when it was computed as percentage of initial motility (58 and 62%) for the two breeds, respectively.**

**In general, the depression in sperm motility occurred during semen processing may have been due to: 1- A decrease in concentrations of**

**naturally existing seminal plasma components. 2- Disturbance in proportional the various electrolytes in the diluted semen, relative to that in the seminal plasma and/or addition of substances having toxic and detrimental action on sperm during semen dilution. 3- Possible effect of cold shock to which some sperms are proportionally subjected during both cooling and freezing in addition to thawing rate (e.g., thawing temperature and thawing duration). A similar assumption was also suggested by EL–Gaafary (1990). Salamon and Maxwell (1995), Maxwell and Watson (1996) and Watson (2000) demonstrated that cold shock starts soon after the semen is cooled to room temperature, although it is more critical for ram semen at temperature below 15°C (Fiser and Fairfull, 1986). It is clear from this investigation that tris-based extender have higher freezability for ram semen as compared to skim milk extenders based on post thawing sperm motility, livability and abnormality .**

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# تأثير نوع المخفف على قابلية السائل المنوى للكباش للتجميد

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**قسم الإنتاج الحيوانى – كلية الزراعة – جامعة المنوفية.**

## الملخص العربي

**تم جمع السائل المنوي من٤ كباش أوسيمي ناضجة (٢-٤ سنوات) بواسطة المهبل الصناعي وتم خلط عينات السائل المنوي التى اظهرت ٨٠% فأكثر حركة تقرمية وتركيز الحيوانات المنوية بها لايقل عن ٣x ١٠ $^{٩}$ /مل ، لدراسة تأثير خمسة مخففات على المقدرة التجميدية للسائل المنوى للكباش. مخففان منهما خاليان من اللبن الفرز (المخفف أ والمخفف ب) وثلاثة مخففات أخرى تحتوى على اللبن الفرز (جـ ، د ، هـ) بنسب متباينة هي ٣٥، ٥٠، ٦٥٪ على الترتيب. وكانت النسبة النهائية للجلسرول ١٤,٥٪ وللتخفيف ١ : ٦ ، وتم إجراء عملية الموازنة علي درجة ٤ – ٥ °م لمدة ثلاث ساعات. وتمت إسالة قصيبات السائل المنوي في حمام مائي درجة حرارته ٤٢ °م لمدة ٣٥ ثانية . تم تقيم النسبة المئوية لحركة الحيوانات المنوية التقدمية ، والحيوانات المنوية الحية والحيوانات المنوية الشاذة بعد مرحلة عملية التخفيف ، والموازنة ، وبعد مرحلة التجميد والإسالة.**

**وقد أوضحت النتائج أن تخفيف السائل المنوي بالمخففان أ ، ب (الخاليان من اللبن الفرز) نتج عنهما وبدرجة معنوية عالية (١%) أعلى نسب مئوية لحركة الحيوانات المنوية التقدمية (٣٥,٦ ٪ ، ٤٢,٥٪) ، والحيوانات المنوية الحية (٣٧,٠ ٪ ، ٤٢,١٪) وأقل نسب للحيوانات المنوية الشاذة (٢٣,٨٣ ٪ ، ١٩,٦٨٪) بعد الإسالة ، وذلك مقارنة بالمخففات جـ ، د ، هـ والتي أعطت بعد الإسالة نسب مئوية أقل لحركة الحيوانات المنوية التقدمية (٣١,٣ ، ٢٣,١ ، ٢١,٣٪) ، والحيوانات المئوية الحية (٣١,٩ ، ٢٥,٤ ، ٢٣,٣٪) و نسب مئوية أعلى للحيوانات المنوية الشاذة (٢١,٥١ ، ٢٤,٩٩ ، ٢٦,٧٤٪) على الترتيب .**

**انخفضت النسبة المئوية لحركة الحيوانات المئوية والحيوية بصورة ملحوظة مع ارتفاع نسبة اللبن الفرز في المخفف بينما ارتفعت النسبة المئوية للحيوانات الشاذة ، وكانت الفروق معنوية بدرجة عالية (١%).**

**حافظ المخفف (ب) على أعلى معدل لاسترداد حركة الحيوانات المنوية التقدمية (٥٨,٦٪) والحيوانات المئوية الحية (٥٨,٦٪) عند مقارنة تلك القيم بمثلها بعد عملية الموازنة للسائل المنوي ، وبلغت هذه القيم ٤٦.٥ و ٤٦.١% ، على التوالى، عند مقارنة تلك القيم بمثلها للسائل المنوي قبل قبل إجراء عليات التجميد ، وكما حافظ على أقل زيادة كلية للحيوانات المنوية الشاذة (١٧,٦٨٪) أثناء مراحل التجميد المختلفة. بينما بلغت هذه المعدلات أدناها بالنسبة حركة الحيوانات المنوية التقدمية و والحيوانات المئوية الحية وأعلاها بالنسبة للزيادة الكلية للحيوانات المنوية الشاذة عند إستخدام المخفف (هـ) والذى يحتوى على أعلى نسبة من اللبن الفرز.**

**نتج عن إجراء عمليتي الموازنة والإسالة للسائل المنوي تدهور في حركة الحيوانات المنوية بنسبة عالية تتراوح بين ٢٨.٨ و ٣٦.٩% بينما بلغت هذه النسب أدناها ( ٥.٦ – ٨.٧%) عند إجراء عمليتي إضافة الجليسرول و الموازنة. وبلغت هذه القيم ٢٨.٥ –٣٣.٤ ، ٥.٥– ٩.٥% بالنسبة للحيوانات المئوية الحية ، ١٢.٣٧–١٥.٧٢ ، ١.٦٨–٢.٧٨% بالنسبة للحيوانات المنوية الشاذة ، على الترتيب . ويستخلص من هذه الدراسة أن مخففات الترس تملك القدرة على تجميد السائل المنوي للكباش بصورة أفضل من مخففات اللبن الفرز.**