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EFFECT OF GELATIN SUPPLEMENTATION ON THE QUALITY AND FERTILITY OF RABBIT SPERMATOZOA PRESERVED AT ROOM OR REFRIGERATOR TEMPERATURE DEGREES

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ABSTRACT

Semen preservation is a main limitation in rabbit artificial insemination. The aim of this study was to investigate the effect of gelatin addition at different levels on the quality and fertility of rabbit semen preserved at room (20-25 ˚C) or refrigerator (5 ◦C) temperature degrees. Semen was collected from 15 APRI bucks aged 1 year with artificial vagina twice/week for 10 weeks. Immediately after collection, ejaculates with 70% or more mass motility were pooled and diluted at a rate of 1:5 in Tris-buffer extender supplemented with 0, 1, 2 and 3% gelatin and each level was divided into two portions, then semen was preserved at room temperature (20-25 ◦C) for the 1st portions and at 5 ◦C for the 2nd ones. Motility, livability and acrosome integrity were evaluated in preserved semen at 0, 6, 12, 24, 48 and 72 hours. Synchronized lacting dose were AI with on day 11 post-partum. Results showed that adding gelatin at level of 2% to Tris-extender for both semen preserved at 20-25 or 5 ◦C increased ($P<0.05$) percentages of progressive motility, livability and acrosome integrity, and decreased abnormality of spermatozoa as compared to the control and other extenders, and spermatozoa kept their qualitative characteristics for 24 h after collection, in order to be used in artificial insemination to receptive does Percentage of motility, livability and acrosome integrity of spermatozoa decreased ($P<0.05$) and sperm abnormality increased ($P<0.05$) by advancing storage time in both semen preserved at 20-25 or 5 ◦C. Does inseminated with fresh semen diluted with Tris-buffer extender supplemented with 2% gelatin yielded the highest kindling rate and litter size. In conclusion, rabbit semen could be preserved at room temperature or at 5 ◦C without affecting its quality by adding 2% of gelatin to Tris-buffer extender to avoid expensive materials, and having an easy way to manage and transport within 24 hours.

Keywords*:* Rabbit, semen, gelatin, preservation, sperm characteristics, fertility.

INTRODUCTION

Artificial insemination (AI) is a biotechnological tool used for genetic improvement spread. It is used in all animal species with many purpose including production planning and control, which is associated to maximize enterprise profitability. Using AI with fresh diluted semen has become a routine procedure in large rabbit farms in many European countries (Sinkovics *et al*., 1983; Roca *et al*., 2000) in order to facilitate reproductive management. In Egypt, AI is still practiced on a very limited scale in some rabbit farms (Zeidan *et al*. 2002).

One of the main constraints is the low storage ability of rabbit semen for prolonged periods with acceptable fertility. Rabbit semen can be

successfully processed and preserved up to 24 h without significant reduction in fertility and fecundity (Alvarino *et al*., 1996). Moreover, fertilizing ability of fresh semen can be maintained at 15 ºC through 48 h or even 72 h (Roca *et al.,* 2000; López-Gatius *et al.,* 2005). The Tris-buffers are commonly used to dilute rabbit fresh semen (Viudes de Castro and Tobias, 1997; Castellini *et al.,* 2000; Roca *et al,.* 2000; Lavara *et al.,* 2005).

In order to enhance sperm motility parameters and fertility when fresh semen is stored up to five days, gelatin has been added to extenders in different species such as rabbit (Nagy *et al*. 2002;López-Gatius *et al.,* 2005), sheep (Yániz *et al.,* 2005) or goat (Salvador *et al.,* 2006). To overcome this problem, Roca *et al*. (2000) compared four Tris-based extenders for the short-term preservation of rabbit semen. Their results suggested that Tris-based extenders were effective for a 2- or 3-day-long preservation. Sinkovics and Tóbiás (1997) reported a commercially available boar semen extender supplemented with gelatin for the short-term preservation and transport of rabbit semen. Using this extender, diluted chilled semen can be transported by mail in a special thermos bottle, making AI available to small-scale farmers as well. Although, it was mentioned by Roca *et al*. (2000) that 15 ˚C is more appropriate to store chilled semen than 5 ˚C, as most of the farmers have only a household refrigerator. Fresh diluted semen has been used but its quality can be maintained only for a short period of time (Roca, *et al*, 2000).

Since, gelatin increases the viscosity of the extender, and viscosity affects the motility parameters of spermatozoa (Hirai *et al*., 1997).

The aim of this study was to investigate the effect of gelatin addition at different levels on the quality and fertility of rabbit semen preserved at room (20-25 ˚C) or refrigerator (5 ˚C) temperatures.

MATERIALS AND METHODS

The present study was carried out at the International Livestock Management Training Center (ILMTC), Sakha, Kfr El-Sheikh governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt; during the period from March to May 2011.

Semen collection:

Semen was collected twice weekly form fifteen new local line of rabbit bucks (APRI, produced by Animal Production Research Institute) using artificial vagina for rabbits. The experimental bucks aged 12 months and weighed 3.5 kg LBW). Semen was collected before feeding at 8.00 a.m. Gel plug was removed immediately after collection of semen and keep at 35-37 ◦C in water bath Only ejaculates with mass motility of 70% or more (12-15 ejaculates for each collection day) for 10 weeks (240-300 ejaculates) were taken immediately to the laboratory and pooled. The pooled ejaculates were diluted with Tris-buffer extender and held in a water bath at 37°C, then divided into four portions. Gelatin was added to the three diluted portions of semen at levels of (1, 2, and 3 g/100 ml extender), while the 4th portion was diluted with extender without gelatin addition (control). Semen was diluted at a rate of 1:5

in heated (37 ◦C) Tris-extender. Each 100 ml of the extender was prepared with Tris (3.208g), citric acid (1.675g), fructose (1.25 g, Glycerol (2%) Egg-yolk (10 ml), streptomycin (0.5 g), lincomycin (0.01) and completed with distilled water), then mixed and kept at 37 ◦C. Each portion of four portions was divided into two sub-portions; the 1st sub-portions were stored at 20-25 ◦C (room temperature), while the 2nd portions were stored at 5 ◦C (refrigerator temperature) for 0, 6, 12, 24, 48, and 72 hours.

Semen evaluation:

Percentages of progressive motility, livability, acrosome integrity and abnormality of spermatozoa were determined in both semen preserved at room and 5^0C for a period ranged from 0-72 h. The percentage of motile spermatozoa (progressive motility) was assessed using research microscope with warmed stage (37°C) under the high power magnification (x400) according to Amman and Hammerstedt (1980). Sperm livability percentage was determined using eosin and nigrosin mixture stain according to Hackett and Macpherson (1965). Live spermatozoa (unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa. Then percentage of live spermatozoa was calculated. Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983). The percentage of acrosome integrity was conducted as indicated by Watson (1975).

Artificial insemination of rabbit does:

About 48 h before artificial insemination, 80 rabbit does (APRI) were s.c. injected with 75 IU PMSG: (Folligon, Intervet, Holland) and ovulation was induced with 100 IU HCG analogue (Suprefact, Hoechst Roussel, Madrid, Spain) given at the time of insemination. Total of 20 does were inseminated by semen extended with each level of gelatin and control semen immediately post-dilution using filled plastic AI gun close to the cervical canal, 11 days after parturition. Pregnancy diagnosis was performed by abdominal palpation on day 12 after AI and parturition was subsequently recorded. All inseminations were conducted by the same person. After parturition, kindling rate, litter size and average kit weight were recorded at birth.

Statistical analysis:

Data, after arcsine transformation of the percentages, were examined by ANOVA using a repeated-measures general linear model to evaluate the effect of gelatin level (0, 1, 2 and 3%), storage time (0, 6, 12, 24, 48 and 72 h) or their interaction. Data of kit performance parameters were analyzed using one way design (ANOVA), while kindling rats were analyzed using Chi-square test. Results were statically analyzed according to Snedecor and Cochran (1982) using SAS programme (1985). When ANOVA revealed a significant effect, values were compared using Duncan's multiple range test (Duncan, 1955). All differences were set at $P<0.05$.

RESULTS

Semen stored at room temperature (20-25 ^{o}C):

Results presented in Table (1) showed that the effect of gelatin supplementation on all sperm characteristics was significant (P<0.05). Adding gelatin at levels of 1 or 2% to Tris-buffer extender significantly (P<0.05) increased percentages of progressive motility, livability and acrosome integrity, and decreased abnormality of spermatozoa as compared to the control and 3% gelatin extenders, regardless storage time, being better with 2% than other extenders.

The percentages of progressive motility, livability and acrosome integrity of spermatozoa significantly (P<0.05) decreased and sperm abnormality significantly (P<0.05) increased by advancing storage time. Although they gradually maintained their functions to the minimum levels up to 72 h. However, percentage of acrosome integrity and sperm abnormality was within the normal range up to 12 h (Table 1).

Table (1): Effect of gelatin supplementation and storage time on rabbit sperm parameters in semen stored at 20-25 ^{o}C.

Item	Sperm characteristics (%)			
	Progressive motility	Livability	Acrosome integrity	Abnormality
Effect of gelatin supplementation (g/100 ml):				
Control	46.83±1.93^{c}	47.57±1.94^{c}	61.93±1.02^{c}	18.45±0.42^{a}
1% Gelatin	55.23±1.79^{b}	55.65±1.75^{b}	67.38±1.03^{b}	15.97±0.37^{c}
2% Gelatin	61.92±1.39^{a}	62.73±1.41 ^{a}	73.30±0.92^{a}	13.90±0.30^{d}
3% Gelatin	45.90±2.26^{c}	46.38±2.25 ^{c}	62.60±0.94^{c}	17.60±0.43^{b}
Effect of storage time (h):				
0	69.00±0.82^{a}	69.58±0.84^{a}	74.20±1.17^{a}	13.68±0.36^{d}
6	64.50±0.94^{b}	64.90±1.04^{b}	71.90±1.18^{b}	14.15±0.34^{d}
12	57.75±1.42^{c}	58.30±1.42^{c}	69.50±1.16^{c}	15.45±0.40^{c}
24	49.60±1.65^{d}	50.15±1.67^{d}	63.60±1.01^{d}	17.78±0.47^{b}
48	41.25±2.03^{e}	42.00±2.04^{e}	61.40±0.95^{d}	18.55±0.44^{ab}
72	32.70±1.72^{f}	33.55±1.73^{f}	58.40±0.95 ^{e}	19.30±0.50^{a}

a, b…..f: Means denoted within the same column for each effect with different superscripts are significantly different at (P<0.05).

The effect of interaction between extender treatment and storage period on all sperm parameters was not significant. In semen with different gelatin levels, similar trend of changes were observed for all sperm parameters with advancing storage time. It is of interest to note that 2% gelatin semen extender showed the highest values at all storage times and spermatozoa kept their qualitative characteristics for 24 h after collection, in order to be used in artificial insemination to receptive does (Fig. 1).

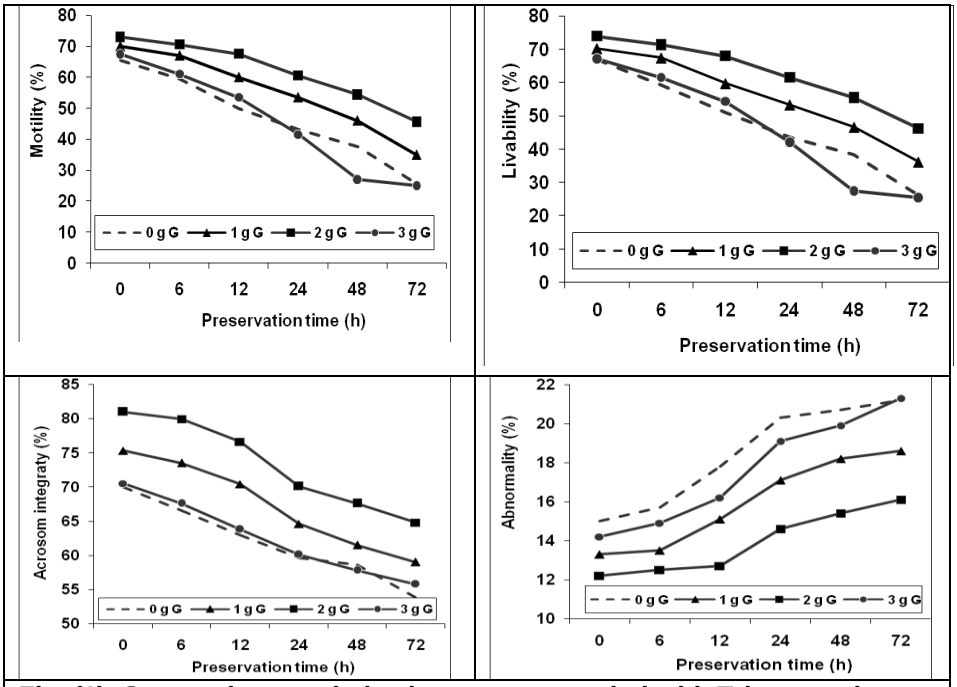

Fig. (1): Sperm characteristics in semen extended with Tris-extenders containing different levels of gelatin and stored at 20-25 ^{o}C.

Semen stored at refrigerator temperature (5°C):

It is worthy noting that all measured parameters in semen stored at 5 ◦C showed similar changes to that observed for semen stored at 20-25 ◦C as affected by gelatin addition and storage time (Table 2) or their interaction (Fig. 2).

Table (2): Effect of gelatin supplementation and storage time on rabbit sperm parameters in semen stored at 5^{0}C.

Item	Sperm characteristics (%)			
	Progressive motility	Livability	Acrosome integrity	Abnormality
Effect of gelatin supplementation (g/100 ml):				
Control	48.30±1.78^{c}	48.97±1.81^{c}	63.42±1.09^{c}	17.48±0.40^{a}
1% Gelatin	55.33±1.95^{b}	55.85±1.91^{b}	70.30±0.93^{b}	15.43±0.30^{b}
2% Gelatin	61.58±1.34^{a}	62.12±1.37^{a}	73.28±0.99^{a}	14.37±0.37^{c}
3% Gelatin	46.33±2.33^{c}	46.90±2.30^{c}	62.57±1.11^{c}	17.43±0.41^{a}
Effect of storage time (h):				
0	69.00±0.82^{a}	69.58±0.84^{a}	74.20±1.17^{a}	13.68±0.36^{d}
6	65.25±0.95^{b}	65.70±0.95^{b}	72.13±1.22^{b}	13.95±0.32^{d}
12	57.75±1.23^{c}	58.00±1.27^{c}	69.78±1.16^{b}	15.40±0.37^{c}
24	50.15±1.63^{d}	50.85±1.66^{d}	66.45±1.08^{c}	16.90±0.37^{b}
48	43.38±1.78^{e}	44.05±1.79^{e}	62.88±1.20^{d}	17.68±0.43^{b}
72	32.00±1.98^{f}	32.58±1.94^{f}	58.93±1.30 ^{e}	19.48±0.42^{a}

a, b…..f: Means denoted within the same column for each effect with different superscripts are significantly different at (P<0.05).

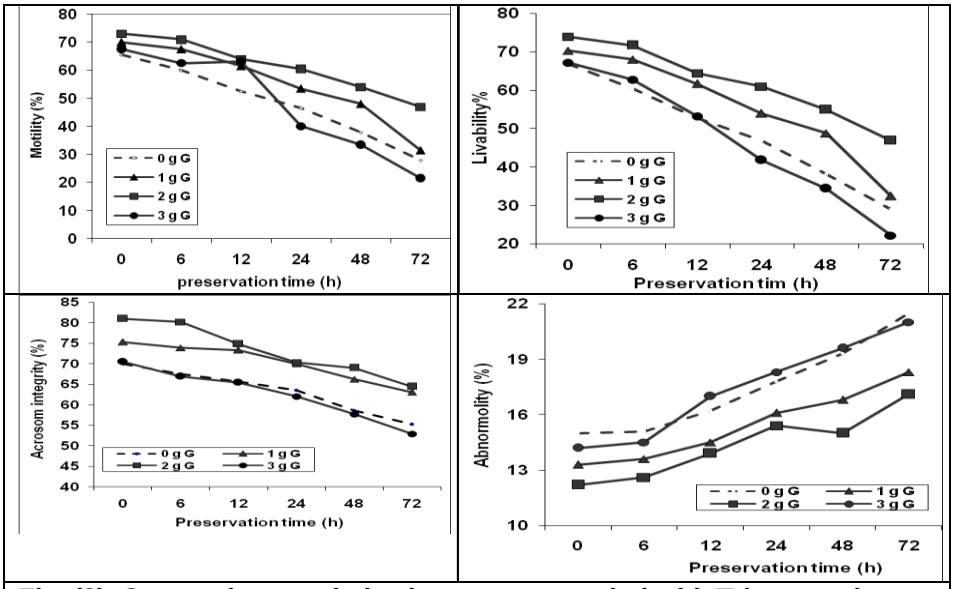

Fig. (2): Sperm characteristics in semen extended with Tris-extenders containing different levels of gelatin and stored at 5 ^{o}C.

Fertility rate and kit performance:

Results presented in Table (3) revealed that insemination of does with 2% gelatin extender yielded the highest kindling rate and litter size as compared to the other extenders.

Table (3): Fertility rate and kit performance of does inseminated with semen supplemented with different gelatin levels:

Item	Doe group			
	Control	1% Gelatin	2%Gelatin	3% Gelatin
Inseminated does	20	20	20	20
Kindling rate (%)	75^{b}	85^{ab}	90^{a}	80^{b}
Litter size at birth	6.67±0.58	7.00±0.411	7.5±0.437	6.8±0.37
Average kit weight (g)	59.3±3.26	65.2±5.77	52.4±4.79	62.4±6.33

a, b and c: Means denoted within the same row with different superscripts are significantly different at (P<0.05).

DISCUSSION

As previously stated, semen preservation is a main limitation in rabbits artificial insemination. One of the main constraints is the low storage ability of rabbit semen for prolonged periods with acceptable fertility. Alvarino *et al*. (1996) reported that rabbit semen can be successfully preserved up to 24 h without deleterious effects on fertility. Other authors indicated that fertilizing ability of fresh semen can be maintained at 15 ºC through 48 h or even 72 h (Roca *et al.,* 2000; López-Gatius *et al.,* 2005). Moreover, rabbit fresh semen can be stored up to five days by adding gelatin to semen

extender (Nagy *et al*., 2002; López-Gatius *et al.,* 2005). The aim of the present study was to investigate the possibility of preservation of rabbit semen for 72 h at room or refrigerator temperature, when different gelatin levels have been added to Tris-buffer extender. The present results revealed that 2% of gelatin extender resulted in significantly (P<0.05) the best percentages of sperm motility, livability, acrosome integrity and normality. In accordance with the present results, López-Gatius *et al.* (2005) found that rabbit sperm motility enhanced when semen diluted with extender supplemented with gelatin.

As affected by preservation period at 20-25 or 5 ◦C in this study, various gelatin extenders (0, 1, 2 and 3%) showed similar trend of deleterious in motility, livability, acrosome integrity and abnormality at different storage times. Similar results were obtained by Zeidan *et al*. (2002) on rabbit semen diluted with different types of extenders and stored at 5 ◦C for two days. The observed gradual reduction in sperm motility by advancing storage period may be attributed to decreasing the content of ATP and this was accompanied by a precipitous fall in rate of fructolysis (Salamon, 1970), while reducing sperm livability and increasing abnormality with advancing preservation time may be due to accumulation of lactic acid, which has a toxic effect on sperm cells and leakage of intracellular enzymes (Zeidan, 1994).

It is of interest to note that semen extended with 2% gelatin had the lowest deleterious rate in sperm characteristics by advancing, showing the best results at all preservation times. In the present study it was determined 48 h as the maximum period of preservation of semen supplemented with 2% gelatin with acceptable percentages of sperm characteristics. In comparable the present results, Torres *et al*. (2004) found that percentages of sperm motility, livability and normality in semen supplemented with gelatin in fresh case or preserved at 15 °C until 4 days were negatively affected by time conservation, however, still 72 h after collection, spermatozoa kept their qualitative characteristics in order to be used in artificial insemination to receptive does. Also, Nagy, *et al*. (2002) reported that gelatin addition had a positive effect in semen quality preserved at 5 °C by 72 h. They found a higher percentage of live cells and acrosome integrity, in semen preserved with gelatin supplemented extender in comparison with no supplemented one. On the other hand, some investigators found no differences in goat and sheep semen motility when fresh semen extender was supplemented with gelatin immediately after semen collection (Yániz *et al.,* 2005; Salvador *et al.,* 2006).

Concerning fertility trial in this study, does artificially inseminated with semen supplemented with 2% gelatin showed significantly (P<0.05) the highest fertility. Based on the foregoing results regard to sperm characteristics and fertility trial, supplementing the extender with 2% gelatin was effective in reducing spermatozoa metabolism and movement. As a consequence of the previous factors, probably there was a reduction of lactic acid generation and it is known that low pH kill the spermatozoa (Torres *et al*., 2004). Also, as gelatin prevents sedimentation, sperm cells are more uniformly distributed and buffers can prevent pH-changes more efficiently

(Nagy, *et al*., 2002). Moreover, gelatin increases the viscosity of the extender, and viscosity affects the motility parameters of spermatozoa (Hirai *et al*., 1997). However, Roca *et al*. (2000) mentioned by that 15 ◦C is more appropriate to store chilled semen than 5 ◦C, as most of the farmers have only a household refrigerator. The observed increase in kindling rate and litter size of does inseminated with semen diluted with extender supplemented with 2% gelatin indicated the positive correlation between motility of fresh semen and fertility as reported by Brun *et al*. (2002) and Lavara *et al*. (2005).

In conclusion, rabbit semen could be preserved at room temperature or at 5 ◦C without affecting its quality by adding 2% of gelatin to Tris-buffer extender to avoid expensive materials, and having an easy way to manage and transport within 48 hours.

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تأثير إضافة الجيلاتين على جودة وخصوبة الحيوانات المنوية للأرانب المحفوظة على درجة حرارة الغرفة أو الثلاجة.

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يعتبر حفظ السائل المنوى من العوامل المحددة للتلقيح الصناعى فى الأرانب. أجريت هذه الدراسة لمعرفة تأثير إضافة مستويات مختلفة من الجيلاتين على جودة السائل المنوى والخصوبة للأرانب بعد ٧٢ ساعة من الحفظ على درجة حرارة الغرفة أو الثلاجة. تم جمع السائل المنوى من ١٥ ذكر أرانب عمر عام من النوع APRI (نوع جديد محلى) باستخدام المهبل الصناعى مرتين أسبوعيا لمدة ١٠ أسابيع (من مارس- أبريل). بعد جمع السائل المنوى تم وضعه على درجة ٣٥ م٠ لحين إجراء الفحص (النسبة المئوية للحيوية، الحى والميت، الشواذ وسلامة الأكروسوم) تم فقط استخدام عينات السائل المنوى التى تزيد حيويتها عن ٧٠% فى التخفيف بمعدل ١:٥ بمخفف الترس مع صفار البيض بإضافة مستويات مختلفة من الجيلاتين (3,2,1,0)جرام/١٠٠ مل مخفف. تم تقسيم كل مستوى إلى جزئيين الأول يوضع على درجة حرارة الغرفة والثاني على درجة حرارة الثلاجة. وتم تقدير النسبة المئوية للحيوية، الحى والميت، الشواذ وسلامة الأكروسوم للسائل المنوى المحفوظ عند الفترات الآتية 0 ,6 ,12 ,24 ,48 ,72 ساعة. تم تقدير القدرة الاخصابية للسائل المنوى المخفف بمستويات مختلفة من الجيلاتين وذلك بتلقيح عدد من إناث الأرانب بعد ١١ يوم من الولادة .

أظهرت النتائج أن إضافة الجيلاتين بمستويات ٢،١ جرام/١٠٠ مل لمخفف الترس مع صفار البيض المحفوظ سواء على درجة حرارة الغرفة أو الثلاجة أدى إلى تحسين النسبة المئوية للحيوية والحى والميت وسلامة الأكروسوم وانخفاض نسبة الشواذ معنويا عند مستوى 5% مقارنة بمجموعة المقارنة وأن إضافة الجيلاتين بمعدل ٢% كان أفضل من ١%. مع طول فترة التخزين قلت النسبة المئوية للحيوية والحى والميت وسلامة الأكروسوم معنويا عند مستوى ٥% أيضا زادت الحيوانات المنوية الشاذة معنويا عند مستوى ٥% سواء على درجة حرارة الغرفة أو الثلاجة.

أظهرت النتائج أن إضافة الجيلاتين بمستوى ٢% أدى إلى تحسين صفات الحيوانات المنوية المحفوظة لمدة ٢٤ ساعة بعد الجمع. أعطى السائل المنوى الطازج المخفف بمخفف الترس مع صفار البيض والمضاف اليه ٢% جيلاتين أعلى خصوبة. الخلاصة أنه يمكن حفظ السائل المنوى على درجة حرارة الغرفة أو الثلاجة دون التأثير على جودة الحيوانات المنوية وقدرتها على الإخصاب بعد إضافة الجيلاتين بمعدل ٢% لمدة ٢٤ ساعة بعد الجمع.

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