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EFFECT OF GELATIN ADDITION TO EXTENDER ON SEMEN QUALITY OF RABBIT

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The effect of gelatin addition to extender on the viability and acrosome integrity of rabbit spermatozoa was studied. Pooled semen samples were processed in a tris based extender with two different levels of gelatin that solidify semen at 5 °C preventing sperm precipitation and unwanted energy expenditure movement. Semen was collected with an artificial vagina twice weekly from seven New Zealand white rabbit bucks. Pooled semen was diluted in tris-based diluted which was provided with 0.8g or 1.6g gelatin(g/100ml extender). Dilution rate was 1 semen: 3 extender. The final concentration rate was 80 – 100 X 10^6 spermatozoa / 100 ml. After storing semen up to 2 days at 5 °C, semen samples were pre-warmed to 25 °C then evaluated with light microscope to reveal the influence of gelatin addition and preservation time (0, 24 and 48 hrs) on sperm motility parameters.

Adding gelatin to the extender improved motility and reduced dead and abnormal spermatozoa and acrosomal damage. The low gelatin content extender improved these parameters, but the high gelatin content extender gave the best results. However, increasing storage time resulted in reducing these parameters. Results showed that gelatin addition had a significant positive effect on the quality of the stored semen which would facilitate commercial distribution.

Key words: Acrosome integrity, extender, gelatin, semen, sperm motility

Artificial insemination in rabbits is usually done with fresh diluted semen (on the day of semen collection), yielding pregnancy rates similar or less than those would be achieved with natural mating (Morrel, 1995). However, attempts are underway to preserve fertility in semen stored for several days. Semen cryopreservation is still a limiting factor for extensive commercial application programs in sheep, horses and rabbits (Curry, 2000). Although frozen-thawed rabbit semen can result in reasonable

kindling rates (Moce *et al.*, 2003), commercial distribution is still of low significance due to semen precipitation and energy expenditure movement during storing. Tris-buffer extenders were effective at preserving fertility for 2 days when spermatozoa were stored at 15℃ (Roca *et al.*, 2000) and the addition of gelatine (1g / 100 ml extender) increased viability and acrosomal integrity of spermatozoa stored for 72h (Nagy *et al.*, 2002).

The aim of this study was to investigate the effect of gelatin addition on the quality of rabbit semen after 0, 24 and 48 hrs preservation at 5 ℃.

MATERIAL AND METHOD

1- Animals:

The experimental work of this study was carried out at Maryout Research Station, Desert Research Center during the period from September 2005 to March 2006. Seven sexually mature New Zealand White rabbit buck (aged12 month and 3 kg body weight). Animals were housed in flat deck cages and fed a commercial concentrate pellet diet containing 16.3% crude protein, 13.3% crude fibre, 2.5% fat, 0.6% mineral mixture and 2600 Kcal/kg digestible energy. Fresh water was made available all day through nipples drinker system. Before start, semen quality (colour, volume, and progressive motility) was assessed and all bucks were selected to have good semen quality and quantity.

2- Semen collection and evaluation:

Semen was collected twice weekly for Twenty weeks from 7 (NZW) buck rabbits with the aid of an artificial vagina. Gel plug was removed immediately after collection. Immediately after collection semen was evaluated and only ejaculates exhibiting active progressive motility percentage (over 60%) were used. Semen from different bucks was then pooled and divided to three equal portions. Each portions was diluted with one of three types of Tris-fructose-yolk extender, one was without gelatine, second and third were containing 0.8 or 1.6g gelatine per 100ml extender, respectively. Addition of extender was at 30℃ and 1:3 semen to extender. The basic extender consisted of 3.786 gm Tris, 2.172 gm Citric acid anhydrous, 5 gm fructose, 12% egg yolk 100000 IU penicillin procaine and 100000 µg streptomycin sulphates according to Zaghloul (2006). Extended semen samples were then cooled to 5℃ in a refrigerator and stored for 0, 24 and 48hrs sperm motility was recorded according to Salisbury *et al.*, 1978. Acrosomal damage was estimated by fixing in a solution of 0.2% glutaraldehyde and integral acrosome percentage was estimated according to Johanson *et al.* (1976). Sperm motility (%) and acrosome damage (%) were estimated at each storage period.

3- Statistically analysis:

Data were statistically analyzed by analysis 0f variance according to Snedecor and Cochran (1982). The differences between means were tested by using Duncan's new multiple range test (Duncan, 1955) by using SPSS (1999).

RESULTS AND DISCUSSION

The effect of gelatin addition and storage on buck semen quality it's presented in Table 1. It could be observed that gelatin addition resulted in the highest sperm motility. The level of 1.6g gelatin gave the highest post cooling sperm motility ($P<0.01$). Gel extended semen samples stored at 5 ℃ were solid (the spermatozoa were immotile). This solid state was clearly observable in all gelatin-stored samples throughout the study. Increasing storage time resulted in decreasing significantly sperm motility. However, adding gelatin reduced the adverse effect of storing time. These results partly agreed with those reported by Nagy *et al.*, (2002).

The lowest sperm abnormality was recorded for 1.6g gelatin/100ml extender (12%) as compared with 0.8g gelatin/100 extender (16%) adds without addition (23%). Abnormal spermatozoa increased with the increase of storage time. Increasing gelatin level improved sperm abnormality state and reduced the adverse effect of storing time. These results are similar to those reported by Cortell and Viudes de Castro (2008).

Data presented in Table 1 showed that the gelatin addition in the extender affected significantly ($P<0.01$) the percentage of acrosomal damage of the cooled rabbit spermatozoa. The least acrosomal damage was noticed at highest gelatin level. Total acrosomal damage was increased with the increasing of storage time. These results are in agreement with those found by Lopez-Gatius *et al.*, (2005).

Data presented in Table 1 showed that, the effect of gelatin addition to cooled extender on the percentage of dead spermatozoa was significantly ($P<0.01$). The lowest beneficial overall mean of dead spermatozoa was at the highest gelatin level. Overall effect of storage time on total dead spermatozoa was 16, 20 and 24% for 0, 24 and 48 hrs, respectively. These results are similar to those reported by Zeidan *et al.*, (2002).

In conclusion, our results showed that there was a positive effect of adding gelatin on the viability and integrity of rabbit spermatozoa after the short-term storage. Our explanation of this finding is that, although buffers were added to the extenders to minimize pH-fluctuations due to the metabolic products of the spermatozoa (Levis, 2000), sedimentation of the sperm cells occur, during preservation. Therefore, pH may be lower at the

Table 1. Mean percentages of bucks semen characters at different levels of gelatin in Tris extender stored at 5?c

Semen characters (%)	Storage time(hrs)	Gelatin level (g/100ml)			Over all means
		0	0.8	1.6	
Motility (%)	**0**	68±1.67	75±2.32	85±1.65	**76±1.01^{A}**
	24	55±3.18	69±1.38	81±1.84	**68±2.80^{B}**
	48	39±1.28	66±1.55	77±1.38	**61±1.89^{C}**
	Overall means	**54±7.11^{c}**	**70±6.38^{b}**	**81±6.15^{a}**	
Abnormal Sperms (%)	**0**	17±1.16	13±1.23	9±1.18	**13±1.15^{C}**
	24	23±1.19	16±2.11	12±2.04	**17±1.91^{B}**
	48	29±2.06	19±1.33	15±1.52	**21±1.13^{A}**
	Overall means	**23±3.48^{a}**	**16±3.14^{b}**	**12±3.25^{c}**	
Acrosomal damage (%)	**0**	**12±2.03**	**8**±1.16	**5**±1.52	**8±1.15^{C}**
	24	16±1.35	11±1.58	7±1.11	**11±1.55^{B}**
	48	20±1.43	14±2.10	9±1.18	**14±1.32^{A}**
	Overall means	**16±2.45^{a}**	**11±4.05^{b}**	**7±3.27^{c}**	
Dead spermatozoa (%)	**0**	21±1.28	16±1.88	12±1.12	**16±1.15^{C}**
	24	26±1.18	20±1.15	15±1.46	**20±1.55^{B}**
	48	31±1.82	24±1.28	18±1.78	**24±1.32^{A}**
	Overall means	**26±3.18^{a}**	**20±2.99^{b}**	**15±3.40^{c}**	

Means with different litters within each item are significantly different (P<0.01).

region of sediment cells. Moreover, the concentration of some toxic metabolic products may be higher at this region. As gelatin prevents sedimentation, sperm cells are more uniformly distributed and buffers can prevent pH-changes more efficiently. In addition, solidification by gelatin reduces sperm movement and lessens energy expenditure.

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تأثير إضافة الجيلاتين إلى المخفف على صفات السائل المنوي للأرانب

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تم أجراء هذه الدراسة لتقيم تأثيرإضافة الجيلاتين إلى مخفف السائل المنوي للأرانب على حيوية الحيوانات المنوية وسلامة مقدم رأس الحيوانات المنوية بعد التخفيف – قسمت عينات السائل المنوي المجمعة في مخفف الترس إلى ثلاثة أجزاء - جزء لا يحتوي جيلا تين (مقارنه) – جزء يحتوي ٠,٨ جرام جيلا تين / ١٠٠ مل مخفف – جزء يحتوي ١,٦ جرام جيلا تين / ١٠٠ مل مخفف ٠

تم جمع السائل المنوي مرتين أسبوعياً ولمدة ٢٠أسبوع بواسطة المهبل الصناعي من سبعة ذكور أرانب نيوزلندي – كل تلقيحه مخففة تحتوي على ٨٠ – ١٠٠ × ١٠^{٦} حيوان منوي نسبة التخفيف ١ : ٣ سائل منوي مخفف على درجة ٣٧°م.

تم تخزين السائل المنوي لمدة يومين على درجة ٥°م ثم تم فحص عينة السائل المنوي على درجة ٢٥°م وذلك بعد صفر– ٢٤– ٤٨ ساعة وقدرة الحركة التقدمية للحيوانات المنوية .

أدت إضافة الجيلاتين إلى المخفف إلى تحسين الحركة التقدمية وخفض نسبة الحيوانات المنوية الميتة والمشوهة والتالف من مقدم الرأس.

استخدام المخفف الذي يحتوي على الجيلاتين بمستوى ٨،.جم /١٠٠مل مخفف حسن الصفات المدروسة بينما المخفف الذي يحتوى ١,٦جم جيلا تين /١٠٠ مخفف أعطى نتائج أفضل بينما إطالة مدة التخزين أدت إلى انخفاض هذه القياسات.

أظهرت النتائج أن إضافة الجيلاتين أعطى معنوية إيجابية على صفات السائل المنوي المخزن.