VIABILITY AND FERTILIZING ABILITY OF EXTENDED RABBIT SEMEN STORED AT 5˚C

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ABSTRACT

A total number of six bucks and sixty five doe rabbits were used in this study to evaluate viability and fertilizing ability of diluted rabbit spermatozoa stored at 5°C. Rabbits were used for semen collection using an artificial vagina. Ejaculates of good initial motility (> 70% motile spermatozoa) were pooled and diluted. Heterospermic pools were divided into three aliquots to study the extender effect on semen motility. In the 1st aliquot semen was diluted in saline solution (0.9% NaCL). The 2nd and 3rd aliquots semen were diluted either with glucose-yolk citrate or fructose-yolk Tris. The dilution rate was 1 semen: 5 diluent v/v. The diluted semen was then cooled to 5°C over 1.5- 2 hours and stored for 2 days. In the fertility trial, sixty five Baladi Black rabbit does were randomly allotted into two groups. Rabbit does of the 1st group were inseminated artificially by fresh semen while those of the 2nd group, were artificially inseminated with cooled semen stored for 24h. All of them were artificially inseminated using a dose of 1 mL containing 50 x 10^6 motile sperm cell. To induce ovulation, each doe was intramuscularly injected with 0.2 ml Receptal/doe.
The effect of the type of extender on the percentage of motile spermatozoa was significant ($P < 0.05$). The highest values of the percentage of motile spermatozoa were recorded with fructose-yolk Tris and glucose-yolk citrate and the lowest with saline solution. The duration of storage time at 5°C up to 48 hours significantly decreased ($P<0.01$) the percentage of motile spermatozoa and sperm viability, irrespective of the extender used. Interactions between the type of extender and storage time on percentage of motile and dead spermatozoa were insignificant. Rabbit doe artificially inseminated with fresh and cooled semen showed similar results. Compared with glucose-yolk citrate, Fructose-yolk-Tris buffer is more effective for dilution and rabbit semen preservation at 5°C, and for maintaining fertilizing ability.

Keywords: Rabbit, semen preservation, extenders, productivity, insemination.

INTRODUCTION

Artificial insemination (AI) technique has become an effective and essential mean for controlling breeding programs not only in experimental scale, but also in commercial rabbittries (Lavara *et al*., 2000, Nizza *et. al.,* 2000 and Rowida, 2003). AI of rabbits is usually done with fresh semen, yielding pregnancy rates similar with natural mating (Morrell, 1995). But AI in rabbits may be accompanied with some detrimental factors affecting fertilizing ability of spermatozoa during preservation. Alvariño (2000) evidenced that rabbit semen preservation is one of the main problems for a wide use of artificial insemination in industrial Rabbitries. Rabbit spermatozoa are more sensible to hypertonic solutions, causing a reduction in storageability and consequently a decrease in kindling rates (Seleem, 2003, Riad *et. al.,* 2004 and Seleem *et al.* Riad, 2005). The use of cooled semen for AI depends upon the ability of the extenders to provide a suitable environment for spermatozoa metabolism. Different buffers have been tested to evaluate their ability to keep chilled rabbit semen during storage (Castellini, 1996). Roca *et al*. (2000) found that Tris-buffer was effective at preserving fertility for 2 days when spermatozoa were stored at 15°C. The aim of the present study was to evaluate the viability and fertilizing ability of rabbit spermatozoa diluted in different extenders and stored for 48 hours at 5°C.

MATERIALS AND METHODS

The present study were carried out at Rabbits Research Unit, Department of Animal Production, Institute of Efficient Productivity, Zagazig University, Zagazig, Egypt, during the initial of September 2009 and lasted 5 months.

In a first experiment, a total number of six Baladi Black bucks rabbits were used. Semen was collected with an artificial vagina once a week. At each collection, ejaculate volume (mL), sperm motility (%), dead spermatozoa (%), abnormal spermatozoa (%), sperm concentration per ml (x 10^6) and total sperm output per ejaculate (x 10^6) were examined microscopically according to El-Gaafary (1987). Ejaculates of good initial motility (> 70% motile spermatozoa) and having a minimum concentration of 200 x $l0^6$ spermatozoa per mL were pooled. Heterospermic pools were divided into three parts to study the extender effect on semen characteristics. The 1st aliquot was diluted with saline solution (0.9% NaCL). The 2nd and 3rd ones were extended with diluent based on glucose-yolk citrate and on fructose-yolk Tris (El-Gaafary (1987). The dilution rate was 1 semen: 5 diluent v/v. Diluted semen was then cooled to 5°C over 1.5-2 hours. Semen quality in term of sperm motility and dead spermatozoa were observed during storage time (0, 12, 24 and 48 hours at 5°C).

In experiment 2, sixty five Baladi Black (BB) doe rabbits were randomly allotted into two groups. Does in the 1st group were inseminated with fresh diluted semen. In the 2nd group, does were inseminated with cooled diluted semen and stored for 24 hours at 5°C. Each experimental group was divided into three sub-groups according to the type of semen extenders. In the 1st sub-group, does were inseminated by diluted semen based on saline solution (0.9% NaCL). In the 2nd sub-group, does were inseminated with an extender containing glucose-yolk citrate (Sodium citrate 80.58 mM, glucose 40.00mM and egg yolk 20%,v/v). In the 3rd sub-group, does were inseminated with diluted semen based on fructose-yolk Tris (Tris 300 mM, fructose 27.75 mM, citric acid 94.7 mM and egg yolk 14%,v/v). All does were inseminated with 1 mL containing 50 x 10^6 motile spermatozoa during three consecutive parities. Before insemination, does were intramuscularly injected with 0.2 mL Receptal (Intervet laboratory). Abdominal palpation was practiced 10 days after AI to detect pregnancy. Conception rate (%), gestation length (d), litter size and litter weight (g) were recorded.

The rabbits were housed individually in wire cages (60 x 55 x 40 cm) provided with galvanized feeders and nipple drinkers. Rabbits were fed isonitrogenous and isoenergetic diets (containing about 17% crude protein and 2700 Kcal ME/kg DM, according NRC, 1977). The diets were offered to rabbits *ad libitum* and fresh tap water was available all the time. The animals were kept under the same environmental and managerial conditions. The light regime used was about 16 h/day (artificial light).

Statistical analysis

Data were statistically examined by analysis of variance (ANOVA) according to Snedecor and Chochran (1982) using SPSS system (2006). The differences between means were tested by using Duncan's New Multiple Range test, (Duncan, 1955). Conception rate was analyzed using the contingency Tables according to Everitt (1977).

RESULTS AND DISCUSSION

1. Semen quality (Experiment 1)

The highest values of the percentage of motile spermatozoa were recorded with fructose-yolk Tris and glucose-yolk citrate and the lowest with saline solution (Table 1). The effect of the type of extender on the percentage of motile spermatozoa was highly significant ($P < 0.05$). These results are in agreement with those of Daader and Seleem (1999) who found that the Fructose-yolk Tris leads to a higher motility compared to a citrate-based extender with bull semen during chilled storage at 5°C for up to 4 days. The lowest values of dead spermatozoa percentage were recorded with fructose-yolk Tris and the highest with glucose-yolk citrate.

Table 1: Effect of type of extenders and storage time on motility and dead spermatozoa.

	Motility (%)	Dead spermatozoa (%)
Extenders effect (A):		
Saline solution	67.50 ± 2.15^{b}	33.12 ± 1.51^{ab}
Glucose-yolk citrate	70.62 ± 2.05^{ab}	43.75 ± 8.88^{a}
Fructose -yolk Tris	71.25 ± 1.78^{a}	28.50 ± 1.38^{b}
Significance	*	*
Storage time effect (B):		
Fresh (0 h)	81.67 ± 1.67^{a}	21.08 ± 0.45^{c}
12 h	73.33 ± 1.55^{b}	33.67 ± 0.46^{b}
24 h	65.83 ± 1.33^{c}	41.67 ± 0.67^{a}
48 h	58.33 ± 1.43^{d}	44.08 ± 2.90^{a}
Significance	**	**
Interaction (A x B)	NS	NS

Means with different superscripts in the same row differ significantly ($P < 0.05$).
** = $P< 0.01$, * = $P < 0.05$ and NS = Not significant

The effect of the type of extender on the percentage of dead spermatozoa was significant ($P < 0.05$). These results are in agreement with those of Zeidan (1994) who found that the effect of different extenders on the percentage of dead bull spermatozoa was highly significant ($P<0.01$).

The present results showed that the storage time at 5°C up to 48 hours significantly decreased ($P<0.01$) the percentage of motile spermatozoa, irrespectively of the extender used. In a same way, storage at 5°C up to 48 hours significantly increased ($P <0.01$) the percentage of dead spermatozoa. Interactions between the type of extender and storage time were not significantly on percentage of motile and dead spermatozoa. These results are in agreement with those of El-Gaafary (1987) and Zeidan (1994). Also, this trend was similar to the results obtained by Riad (2003) and Riad *et al.* (2004). The observed reduction in semen quality when the duration of the storage increases may be due to the increase in lactic acid accumulation as a result of sperm anaerobic metabolism leading to changes in both the osmotic pressure and pH of the media, which might exert a toxic effect on the sperm cells (Zeidan, 1994 and Riad, 2003).

Rabbit semen has been successfully stored at liquid state for short periods of time at 5°C (6 hours for fresh and 48 hours for refrigerated semen) without serious loss of its fertilizing ability (Daader and Seleem, 1997; Zeidan *et. al.*, 2002 and Seleem *et al.*, 2005). The storage duration of extended rabbit semen, significantly decreased motility, spermatozoa viability and acrosomal damages, as well as the chemical characteristics. (Seleem *et al.*, 2005).

Tris-buffer extenders have been found to be useful in short-term storage (6 hours) of chilled rabbit semen (Maertens and Luzi, 1995). However, experiments to evaluate their use in prolonging sperm viability and fertility throughout time are very limited. El-Gaafary (1994) using a Tris-yolk extender found that spermatozoa cooled and stored at 5°C for 24 hours had a motility of 45% and after 48 hours dropped to 25%. This sharp decline in sperm viability throughout the preservation time might be due to chilling temperature. Roca, *et al*. (2000) demonstrated that 15^{0}C can be adequate temperature to store chilled rabbit semen when tris-buffer extenders are used.

2. Fertility trait (Experiment 2)

Cooled semen preservation does not significantly depress the conception rate and the litter size at birth, and at 21 or 28 days (Table 2). Pregnancy length was significantly higher for cooled semen (30.8 vs 30.5 days, $P<0.01$). The results also indicated that most offspring traits (kits weight at birth, 28 days, litter weight gain and pre-weaning mortality) were not affected by using cooled semen (Tables 3 and 4). Mortality at birth was lower with cooled semen (6.58 *vs* 16.93 %) but significantly higher the last week before weaning (6.14 vs 1.01 %). The glucose-yolk-citrate significantly depressed fertility (57.5 *vs* 66.7 and 70.0% respectively, for saline solution and Fructose-yolk-tris) and increased the gestation length without affecting offspring growth nor pre-weaning viability. In North Africa, AI of rabbits is usually done with fresh diluted semen (on the day of semen collection), yielding pregnancy rates similar to those achieved with natural mating (Morrell, 1995). Tris-buffer extender was effective at preserving motility for 2 days when spermatozoa were stored at 15°C (Roca *et al*., 2000 and Lopez-Gatius *et al*. 2005).

Table 2: Effect of type of semen preservation and type of extenders on some fertility traits of rabbit does

Items	No of inseminated does	No of pregnant does	Conception rate (%)	Gestation length (days)	Litter size at		
					Birth	21 days	28 days
Type of semen effect (A):							
Fresh semen	122	78	63.9	30.45±0.06^{b}	6.13± 0.28	5.08±0.28	5.03±0.28
Cooled semen	58	38	65.5	30.84±0.06^{a}	5.79±0.035	5.32±0.33	5.11±0.35
Significance			NS	**	NS	NS	NS
Extenders effect (B):							
Saline solution	54	36	66. 7^{a}	30.53±0.09^{b}	6.12±0.43	5.35±0.47	5.29±0.46
Glucose-yolk-citrate	66	38	57.5^{b}	30.74±0.07^{a}	6.00±0.32	4.95±0.27	4.84±0.30
Fructose-yolk-Tris	60	42	70.0^{a}	30.48±008^{b}	5.95±0.39	5.19±0.38	5.05±0.37
Significance			*	*	NS	NS	NS
Interaction (A x B)			NS	*	NS	NS	NS

Means with different superscripts in the same row differ significantly (P < 0.05).
** = P< 0.01, * = P < 0.05 and NS = Not significant

Table 3: Some productive traits related to AI with fresh or cooled semen, using different extenders

Items	Litter weight at			Kits weight at			Litter weight gain from		
	Birth	21 days	28 days	Birth	21 days	28 days	Birth-21days	Birth-28 days	21-28 days
Type of semen effect (A):									
Fresh semen	320±14	1393±71	2300±112	55±1	285± 8^{b}	486± 13	1135±58	1980±102	907±51^{a}
Cooled semen	308±15	1595±82	2305±122	56±2	232± 16^{a}	483± 15	1287±74	1997±113	711± 64^{b}
Significance	NS	NS	NS	NS	*	NS	NS	NS	*
Extenders effect (B):									
Saline solution	322±24	1471±13	2294±174	54±1^{ab}	295± 20	477± 26	1310±82	1972±155	824±79
Glucose-yolk-citrate	310±15	1429±78	2274±110	53±1^{ab}	291± 6	493± 12	1119±71	1963±100	845± 51
Fructose-yolk-Tris	317±16	1480±92	2333±156	59±3^{a}	305± 13	484± 15	1163±82	2017±146	854± 80
Significance	NS	NS	NS	*	NS	NS	NS	NS	NS
Interaction (Ax B)	NS	*	NS	*	NS	NS	*	NS	NS

Means with different superscripts in the same row differ significantly (P < 0.05).
* = P < 0.05 and NS = Not significant

Table 4: Effect of type of semen and type of extenders on pre-weaning mortality percentage.

Items	Pre-weaning mortality (%)		
	Birth	21 day	28 days
Type of semen effect (A):			
Fresh semen	16.93^{a}	17.69	1.01^{b}
Cooled semen	6.58^{b}	11.62	5.14^{a}
Significance	**	NS	**
Extenders effect (B):			
Saline solution	15.69	16.42	0.89
Glucose-yolk-citrate	14.86	16.54	3.95
Fructose-yolk-Tris	10.44	12.46	2.18
Significance	NS	NS	NS
Interaction (A x B)	*	NS	NS

Means with different superscripts in the same row differ significantly (P < 0.05).
** = P< 0.01, * = P < 0.05 and NS = Not significant

CONCLUSION

Compared with glucose-yolk citrate, Fructose-yolk-Tris buffer is more effective for dilution and rabbit semen preservation at 5°C, and for maintaining fertilizing ability.

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