Effect of semen extender components on rabbit sperm motility

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Abstract

Three different rabbit semen extenders based on Tris-egg yolk extender were used in this experiment. To examine the effects of 1% glycerol or 6% dimethylsulfoxide (DMSO) and control (free of glycerol and DMSO) on rabbit sperm motility and acrosomal changes % over a period of three days of storage at 5 °C. Semen samples from White New Zealand (WNZ) bucks were evaluated for initial concentration, pH value and volume in relation to sperm motility and acrosome loss % . The obtained results showed that, there were significant differences ($p < 0.05$) according to extender type used. Where sperm motility % were 38.0 ± 4.5, 47.3 ± 3.8, and 37.9 ± 4.1 % as for control, 1 % glycerol and 6 % DMSO extender, respectively. The correlated studied characters of acrosomal loss % for the same extenders differed significantly ($p < 0.05$) and were 8.5 ± 0.63, 4.5 ± 0.33 and 7.6 ± 0.68 % respectively.

Key Words: Sperm, Rabbit, Glycerol, Dimethylsulfoxide, *Cryoprotectants*

Introduction

Many research articles were conducted to study the effects of glycerol addition in rabbit semen extenders on both sperm motility and acrosome integrity (Weitze, 1977 and Weitze *et al.,* 1982). The most popular theory for the beneficial effect of glycerol addition in semen extenders is protection impact of glycerol through salt-buffering mechanism (Lovelock, 1953; Polge and Soltys, 1957). Others reported that glycerol can penetrate sperm cell membrane and concentrate in the posterior part of the sperm head (Pickett and Merilan, 1957).

The recent studies tried to prove that some cryoprotective agents like glycerol have toxic effect on rabbit sperm survival through its content of hydroxyl groups (Hanada and Nagase, 1980). Other authors stated that cryoprotectants containing amides or methyl groups like DMSO have been recommended and used successfully for rabbit sperm preservation (Arriola, 1982; Chen and Foote, 1988).

The objectives of this study were to investigate the effect of cryoprotectants like glycerol and DMSO on rabbit sperm motility and acrosome changes in relation to essential semen characteristics in cooled preserved rabbit semen at 5 °C.

Materials and Methods

Bucks and semen collection:

Three New Zealand White (NZW) bucks were used in this experiment with an average body weight 2.5 – 3.5 kg and 12 months of age. The bucks were housed in a wire cages and the doe was transmitted to buck's cage for semen collection. Semen samples were collected by using a glass artificial vagina, supported with a graduated collecting tube and the added water was adjusted on 45 °C at the time of semen collection (Morrel, 1995).

Semen sample evaluation:

The collected semen samples were put in a water bath adjusted at 38 °C. The samples were subjected to the following examinations: general appearance, sample volume, sperm concentration / ml, pH value and initial motility. Sperm motility was estimated by using a light microscope at 100x magnification on a stage warmed at 38 °C. Semen samples less than 60 % motility were discarded and not included in the experiment.

Semen extender components:

Tris-egg yolk extender was prepared according to Fischer and Odenkirchen, 1988. Tris-egg yolk extender contained 360 mM Tris, 33.3 mM glucose and 113.7 mM citric acid for control treatment. The second treatment contained 1 % glycerol in the Tris-egg yolk extender. While the third treatment contained 6 % (vol. / vol.) dimethylsulfoxide (DMSO) in Tris-egg yolk extender. For all treatments egg-yolk, penicillin and streptomycin were added to give a final concentration of 5 % (vol. / vol.), 0.01 % (wt / vol.) and 0.05 % (wt / vol.) respectively.

Semen extension and storage:

The evaluated semen samples were diluted (1 : 5) with three different treatments as mentioned previously at room temperature. The diluted semen samples were put in a closed test tubes (5 ml) in a refrigerator at 5 °C. The storage period of the diluted semen samples was 3 days. To achieve required cooling temperature the tubes were put in a water bath provided with a thermometer to check the required cooling temperature (5 °C) in the water bath every day of the storage period.

Sperm motility and acrosomal changes assessment:

Initial motility was recorded for both raw collected semen and diluted samples directly after semen collection and during three successive days of storage period at 5 °C by using light microscope with 100x magnification. As for acrosome changes determination, two smeared slides of diluted semen samples were prepared directly after dilution and daily during the storage period. The dried smeared slides were examined directly without staining for acrosomal changes according to Watson and Martin, 1972 by using oil lens of light microscope to obtain 1000x magnification. A total count of one hundred sperm cells was examined for the acrosomal change % for each slide. The average of two slides was recorded for each determination.

Statistical analysis:

Analysis of variance (ANOVA) was carried out using starting spss/pc program (1993) to study the effects of semen extender ingredients and storage period on both sperm motility % and percentage of acrosomal loss of different semen samples. Correlation was estimated between raw semen characteristics and each of sperm motility % and acrosomal changes % and between sperm motility % and acrosome changes %.

Results and Discussion

Sperm motility examination:

Results presented in Table 1 showed that, the sperm motility % was superior and differed significantly (P < 0.05) as for Tris-egg yolk extender contained 1 % glycerol which was 47.4 ± 3.8 % for the entire storage period of three days. Whereas, sperm motility % of control treatment (Tris-egg yolk free of glycerol and DMSO) and Tris-egg yolk extender contained 6 % DMSO were 38.0 ± 4.5 and 37.9 ± 4.1 %, respectively for the same storage period. These significant differences may be due to the effect of glycerol in avoiding or controlling cold shock when sperm cells are preserved at 5 °C. It is obvious that rabbit sperm is sensitive and may be died by chilling at temperatures above freezing points as declared by Walton, 1957 and for other animal species by Kumar et al., 1994; Singh et al., 1994; Katila, 1997. The beneficial effect of glycerol which was achieved at this added concentration (1 %) may be associated with the non-toxic effect of glycerol to rabbit sperm especially at this level. The useful effect of glycerol addition was not achieved in the case of DMSO, which can demonstrate the unnecessary to add DMSO alone as a cryoprotective agent in rabbit semen extenders for cooling preservation. Other authors found that cryoprotectants containing amides or methyl groups have been recommended and used successfully for frozen semen (Hanada and Nagase, 1980; Arriola, 1982). Sperm motility percent varied significantly (P < 0.05) according to storage period for different treatments (Table 1). The addition of 1 % glycerol in Tris-egg yolk extender maintained sperm motility significantly differed as compared to control or DMSO treatments (Table 1). It is obvious that both of glycerol and DMSO addition maintained sperm motility in a relatively good condition comparing to control treatment during storage period especially glycerol treatment at third day of storage period (Table 1). Also there was a sharp decrease in sperm motility after first day of storage period for all treatments (Table 1) especially control and DMSO treatments. The explanation of this phenomena may be due to high sensitivity of rabbit sperm to face cooling preservation.

Acrosomal changes assessment:

Studying the acrosomal loss percentage showed that, there were significant differences (P < 0.05) among different treatments. The acrosomal changes % were 8.5, 4.5 and 7.6 for control, 1 % glycerol and 6 % DMSO extenders respectively for complete storage period at 5 °C (Table 2). The addition of 1 % glycerol to Tris-egg yolk extender was significantly increased (P < 0.05) preservation of acrosomal integrity across storage period as compared to the other treatments during three successive days of cooling as shown in Table 2. There were different stages of acrosomal loss. The observed acrosomal status was recorded to be five different stages as shown in Figure 1. To distinguish between different stages of acrosomal loss, shape No. of sperm cell considered to be intact acrosome for the typical complete or non-changed acrosome, while shapes from No. 2 until No. 5 considered to be acrosomal changes (Fig. 1). There are many changes can occur to sperm cell at acrosomal level during cooling preservation. One of this alterations is the spatial arrangements of the internal structure and outer covering of the cells. Beside a contraction of protoplasm may occur and can happen at different rates depending on the chemical components of the various structures (Slisbury, 1978) including acrosomal structures. The beneficial effect of glycerol at this level in this experiment (1%) may be come from the ability of glycerol to reduce mechanical destructiveness to rabbit sperm cells at acrosomal level.

Semen samples characteristics:

Values of semen sample evaluation as regard volume, pH value and initial concentration presented in Table 1. There was positive correlation between these characters and sperm motility as shown in Table 3. In addition there were negative relations between sperm motility % and value of pH as shown in Table 3. In addition there was negative relationship between sperm motility and acrosomal changes % especially for first two days of cooling preservation as for glycerol and control treatments as shown in Table 4. But this relation did not persist as for third day of storage where there was a positive relationship between sperm motility and acrosome changes %. These relations may explain the importance of initial semen evaluation for the samples that will process for refrigeration storage.

It could be concluded, that rabbit sperm is sensitive to great extent to cooling preservation. The extenders, which are used, must contain a cryoprotectant agent like glycerol but in a low concentration to avoid toxicity of rabbit sperm (1 % glycerol could be recommended). Also to obtain a moderate rate of rabbit sperm motility, it is useful to store rabbit semen for only one day at 5 °C. Beside, the technique of acrosomal loss % determination using oil lens of light microscope without any kind of staining is a rapid and simple method for assessing acrosomal changes during storage.

Table 2. Mean ±SE of sperm acrosomal changes percent of different semen extenders during storage period at 5 °C.

Sample No.	Sperm Acrosomal Loss %											
	Control				Glycerol				DMSO			
	A.D	1st	2nd	3rd Day	A.D	1st	2nd	3rd Day	A.D	1st	2nd	3rd Day
1	3	8	12	12	2	5	5	7	2	5	8	13
2	4	6	10	10	4	3	5	6	4	4	8	12
3	2	7	9	10	2	3	7	8	3	6	9	14
4	4	8	10	11	3	4	4	5	4	5	7	12
5	5	7	11	13	4	3	4	6	4	6	10	11
6	4	8	12	14	2	4	6	7	5	5	9	14
7	6	9	11	13	4	2	5	7	4	6	10	12
Mean ±SE	4 .49	8^{A} .37	11^{D} . 42	12^{G} .59	3 .38	3^{B} .37	5^{E} .4	7^{H} .37	4 .36	5^{C} .29	9^{F} .42	13^{G} .43
Overall Mean ±SE	8.5^{A} 0.63				4.5^{B} 0.33				7.6^{A} 0.68			

Means with the same letters showed no significant differences using Duncan multiple range test.

Table 3. Correlation among raw semen characters, sperm motility & sperm acrosomal loss%

Correlation	Raw Sperm Conc.			Value of pH			Sample Volume		
1st day sperm motility (Control) (Glycerol) (DMSO)	0.56	0.64	0.63	-0.66	-0.47	-0.7	0.83**	0.66	0.6
2nd day sperm motility (Control) (Glycerol) (DMSO)	0.90**	0.90**	0.77*	-0.68	-0.58	-0.4	0.80*	0.55	0.5
3rd day sperm motility (Control) (Glycerol) (DMSO)	0.91**	0.86**	0.63	-0.46	-0.37	0.0	0.42	0.47	0.0
1st day acrosomal loss (Control) (Glycerol) (DMSO)	0.30	-0.37	0.50	0.34	0.76*	-0.	-0.25	-0.73	0.
2nd day sperm motility (Control) (Glycerol) (DMSO)	0.12	0.67	0.67	0.29	-0.37	-0.	-0.53	0.71	0.4
3rd day sperm motility (Control) (Glycerol) (DMSO)	0.5	0.68	0.55	-0.09	-0.29	-0.	-0.28	0.63	0.

**P < 0.01

* P < 0.05

Table 4. Correlation between sperm motility and acrosmal changes % during storage period.

Correlation	Acrosomal changes %
1st day sperm motility (Control) (Glycerol) (DMSO)	-0.46 -0.23 -0.5
2nd day sperm motility (Control) (Glycerol) (DMSO)	-0.27 0.78 0.35
3rd day sperm motility (Control) (Glycerol) (DMSO)	0.44 0.82** 0.72

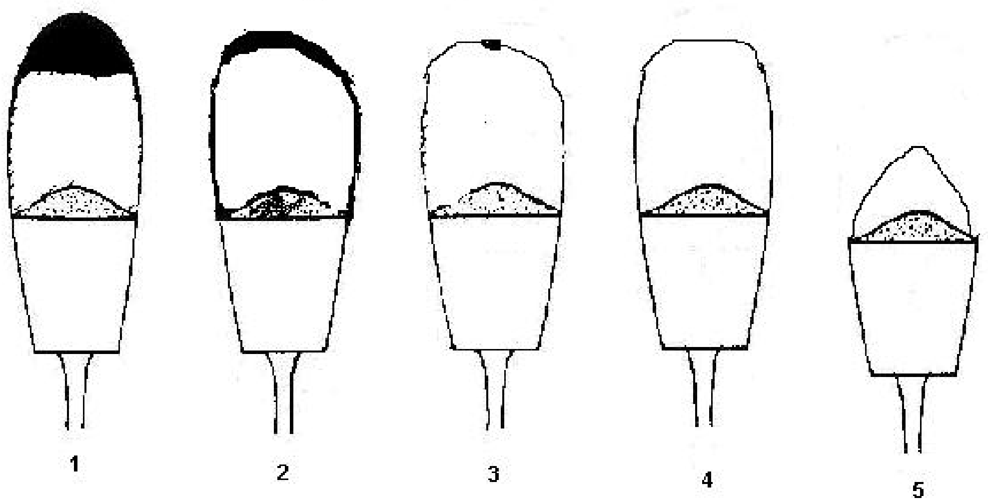

Figure1. Different stages of acrosomal changes of rabbit sperm during cooling storage. No. 1 considered normal acrosome while No. 2 to No. 5 are changed acrosome when examined by using oil lens of light microscope(1000x magnification).

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