JRVM
Journal of Research in Veterinary Medicine

RESEARCH ARTICLE

J Res Vet Med. 2022: 41 (2) 88-91
DOI:10.30782/jrvm.1125759

Effect of Extenders Including High Concentrations Dimethyl Sulfoxide (DMSO) on Post-Thaw Rabbit Sperm Parameters

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Received 03-06-2022 Accepted 05-09-2022

Abstract

Cryoprotectants have critical roles to prevent cell damages during cryopreservation. However, the adjustment of cryoprotectant concentration is also very crucial to protect cells from cryoprotectant toxicity. The present study was designed to investigate the effect of extenders including high concentration dimethyl sulfoxide (DMSO) on post-thaw rabbit sperm quality. Pooled rabbit semen samples (n=7) were diluted and cryopreserved in extenders including 250 mmol/L Tris, 88 mmol/L citric acid, 47 mmol/L glucose, 1% sucrose and different concentrations of DMSO (8%, 10%, 12% and 14%). The presence of high concentration DMSO (12% and 14%) in extender decreased sperm total and progressive motility ($P < 0.01$). The 8% and 10% DMSO supplementations in extender increased live spermatozoon rates ($P < 0.01$). Live and intact acrosome or intact membrane spermatozoon rates were detected higher in 8 and 10 DMSO groups ($P < 0.05$). Although total intact membrane spermatozoon rates were similar in all groups, total intact acrosome spermatozoon rate was higher in 8 DMSO group compared to 12 DMSO group ($P < 0.05$). In conclusion, when 12% and 14% DMSO additions in extender adversely affected post-thaw sperm parameters, the presence of 8% DMSO in extender provided the highest post-thaw sperm quality.

Keywords: sperm, rabbit, cryopreservation, dimethyl sulfoxide.

Introduction

Recently, rabbit production has gained importance as an alternative meat sources in many countries. Thus, assisted reproductive techniques such as sperm cryopreservation[1], artificial insemination[2], etc. have started to study and use more commonly in this species. Unfortunately, the rabbit sperm freezing technology has not reached a sufficient level compared to farm animals, yet. Therefore, different cryoprotectants[3], proteins[4], sugars[5] and antioxidants[6] were supplemented in freezing extenders to improve post-thaw rabbit sperm quality. The cryoprotectants are the most important components of freezing extenders. Either permeable (glycerol, dimethyl sulfoxide (DMSO), ethylene glycol) or non-permeable (saccharides and lipoproteins) cryoprotectants are supplemented in cryopreservation extenders to freeze semen of various animals.[7] When permeable agents bind intracellular water and suppress the production of ice crystals, non-permeable agents enhance extracellular osmolality and trigger cell dehydration.[8] Although both glycerol and DMSO are common cryoprotectants, glycerol is not proper for freezing of rabbit sperm due to its low water permeability and high activation energy.[9] Additionally, DMSO was found more efficient than dimethylacetamide (DMA) for the freezing of the rabbit sperm.[10] Iaffaldano et al.,[10] also reported that 8% DMSO supplementation in freezing extender provided better post-thaw sperm quality than 4% and 6% DMSO supplementations. The post-thaw motile and membrane intact sperm rates were found higher in presence of 10% DMSO when compared with 5% DMSO.[3] The previous studies have indicated that sperm freezing extenders including 8% and 10% DMSO might be good option for rabbits.[3,10] However, the effect of higher concentrations of DMSO on post-thaw

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rabbit sperm parameters has been needed to search. Thus, the present study was designed to investigate the effect of extenders including high concentrations dimethyl sulfoxide (DMSO) on post-thaw rabbit sperm parameters.

Materials and Methods

Animals, Sperm Collection and Cryopreservation

The present study was approved by the Ethical Committee of Aydın Adnan Menderes University (64583101/2022/010). A total of five mature White New Zealand rabbits were used as sperm donors in this study. Semen samples were collected with artificial vagina. Collected semen samples were pooled after initial semen evaluation. The semen samples were higher than 70% motility and less than 25% abnormal sperm rate were used for pooling process. The pooled semen samples (n=7) were separated four equal groups. The sperm samples belongs to each group were diluted at a ratio of 1:3 (to a final concentration of approximately 120 × 10^6 spermatozoa per ml) in a sperm freezing extender including 250 mmol/L Tris-hydroxymethylaminomethane, 88 mmol/L citric acid, 47 mmol/L glucose, 1% sucrose[10] and different concentrations of DMSO (8%, 10%, 12% and 14% DMSO groups). The final DMSO concentrations of the groups were respectively adjusted to 8%, 10%, 12% and 14% DMSO with two-step dilution technique. After dilution process, sperm samples were filled in 0.25 mL straws (IMV Technologies) and then cooled from 36 °C to 4 °C in 90 min with a programmable incubator (Nüve ES 120). The straws were incubated further 10 min at 4 °C for equilibration. Eventually, the cooled straws were frozen with liquid nitrogen vapor 6-7 cm above the surface for 10 min, just before directly plunging into the liquid nitrogen.[11] The frozen sperm samples were stored in liquid nitrogen until the start of sperm analyses.

Evaluation of Sperm Motility Parameters by CASA

A phase contrast microscope (Olympus CX 41) connected to the computer assisted sperm analyzer system (SCA®-Sperm Class Analyzer, Microptic) were used to evaluate total and progressive motility parameters. Thus, 3 µL sperm sample from each experimental group was placed on a pre-warmed (37 °C) glass slide for the evaluation. Three different fields from each slide was assessed at 100× magnification by the system.[11]

Evaluation of Sperm Viability and Acrosome Integrity

The sperm viability and acrosome integrity were assessed with propidium iodide (PI) and Lectin from Arachis hypogaea (peanut) fluorescein isothiocyanate conjugate (FITC-PNA) staining procedure.[11] Briefly, 50 µL sperm sample was incubated and stained with 2.5 µL PI (500 µg/mL) and 5 µL FITC-PNA (200 µg/mL) for 10 min at room temperature. The stained 3 µL sperm sample was placed on slide and covered with cover slip. The image of spermatozoa in same field were captured and evaluated with epifluorescence microscope (Olympus BX53) equipped with a differential interference contrast (DIC) and multiple-fluorescence filters (DM505, U-FBW, BA510IF, BP460-495) and epifluorescent optic (×400 magnification) using a digital camera (DP26- Olympus 5.0 MP). Spermatozoa were classified in four categories: live spermatozoon with intact acrosome (no fluorescence), dead spermatozoon with intact acrosome (only red fluorescence), live spermatozoon with damaged acrosome (only green fluorescence) and dead spermatozoon with damaged acrosome (green and red fluorescence together).

Evaluation of Sperm Membrane Integrity

To evaluate sperm membrane integrity hypo-osmotic swelling test (HOST) in combination with eosin-nigrosin (EN) staining procedure was used.[12] Thus, semen sample (25 µL) were added to 475 µL fructose solution (100 mOsm/L). The samples were incubated in 35°C water bath for 20 min. The samples were stained with EN stain and the membrane integrity of the spermatozoon was determined with under bright field microscope at 400× magnification. Spermatozoa were classified in two groups: live-intact (unstained sperm heads and coiled tails) and total intact (stained and unstained sperm heads with coiled tails).

Statistical Analyses

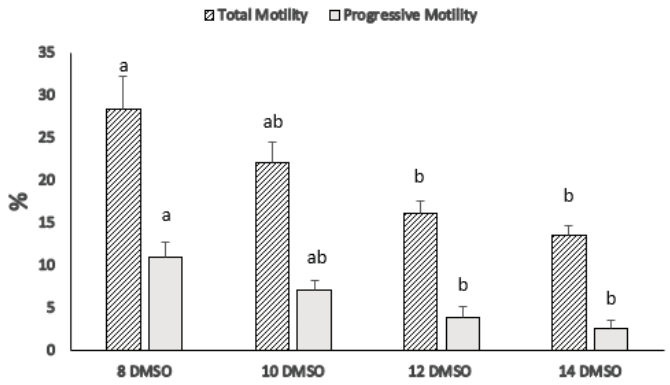
The results of present study (sperm motility, viability, acrosome and membrane integrity) were analyzed with ANOVA General Linear Model (IBM, SPSS Statistics). Significance was determined by fixing probability level at $P < 0.05$. When ANOVA found a significant difference between groups, groups were compared with pairwise multiple comparison post hoc test (Tukey). All the results were presented as mean ± S.E.M.

Results

The total and progressive motility results were higher in 8 DMSO group in comparison with 12 DMSO and 14 DMSO groups ($P < 0.01$) as well as there was no significant difference between 10 DMSO group and other groups (Figure 1). The 8 DMSO and 10 DMSO groups had higher live sperm rates than 12 DMSO and 14 DMSO groups (Figure 2, $P < 0.01$). Similarly, live and acrosome-intact sperm rates of 8 DMSO and 10 DMSO groups were higher than 12 DMSO and 14 DMSO groups (Figure 3, $P < 0.01$). Eight DMSO group had higher total acrosome-intact spermatozoon rate compared to the 12 DMSO group ($P < 0.05$), there was no significant difference between other groups (Figure 3). The live and membrane-intact sperm rate was better in 8 DMSO group than 12 DMSO and 14 DMSO groups (Figure 4, $P < 0.01$). Additionally, 10 DMSO group

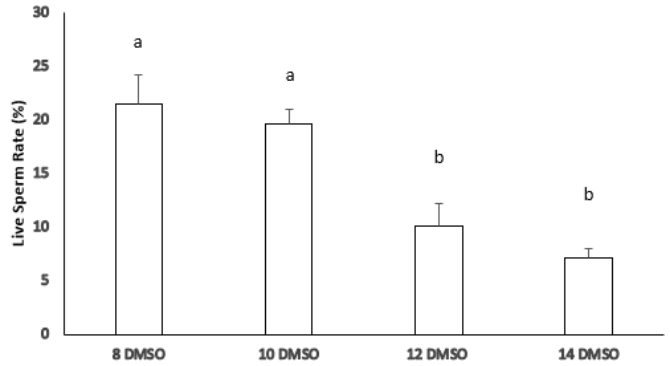
provided higher live and membrane-intact sperm rate than 14 DMSO group ($P < 0.05$). Finally, the total membrane-intact spermatozoon rates were similar amongst all the groups (Figure 4).

Figure 1. Effect of different DMSO concentrations on post-thaw motility parameters of rabbit semen.



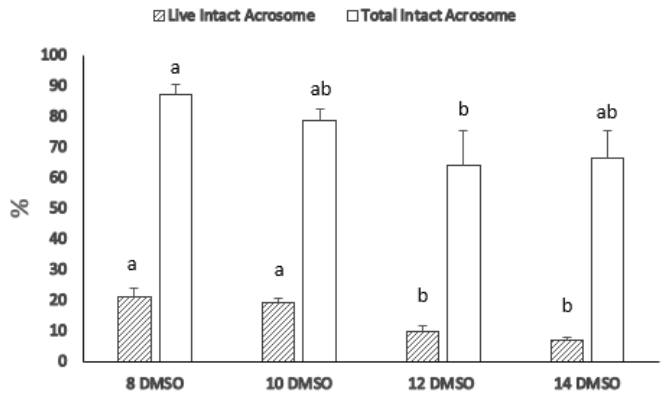
Different superscripts a, b indicates significant differences between groups ($P < 0.01$).

Figure 2. Effect of different DMSO concentrations on post-thaw viability of rabbit semen



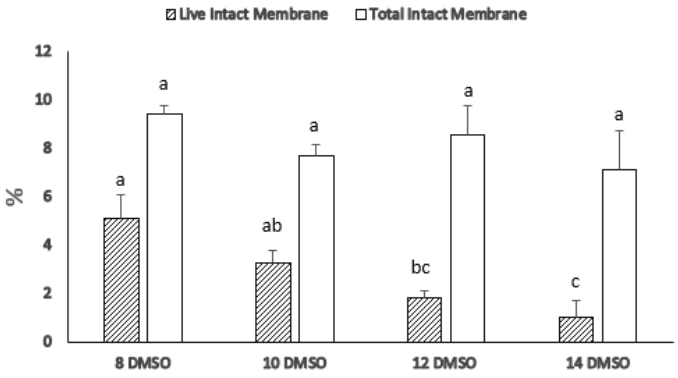
Different superscripts a, b indicates significant differences between groups ($P < 0.01$).

Figure 3. Effect of different DMSO concentrations on post-thaw acrosome integrity of rabbit semen.



Different superscripts a, b indicates significant differences between groups ($P < 0.05$)

Figure 4. Effect of different DMSO concentrations on post-thaw membrane integrity of rabbit sperm



Different superscripts a, b, c indicates significant differences between groups ($P < 0.05$).

Discussion and Conclusion

Cryopreservation technology is an important tool for storage and transfer of sperm cells and subsequently to improve the livestock production and genetic capacity.[13] Although cryoprotective agents are necessary to minimize injuries during cryopreservation process, high concentrations of these agents cause toxic effects on cells.[13] Eight or 10 percent DMSO supplementations were found more beneficial than less concentration of DMSO for rabbit semen cryopreservation in previous studies.[3,10] The effect of higher DMSO concentrations on rabbit semen freezing technology were evaluated in the present study. The present data indicated that the addition of high concentrations of DMSO (12% and 14%) in freezing extender dramatically decreased post-thaw rabbit sperm quality (Figure 1, 2, 3 and 4). Briefly, the high concentration DMSO (12% and 14%) supplementations in freezing extender decreased both total and progressive motility parameters compared to the presence of 8% DMSO (Figure 1, $P < 0.01$). Similarly, Si et al.,[14] reported that 15% DMSO treatment decreased sperm motility compared to 10% DMSO treatment in monkeys. The better motility results in lower concentrate (8% and 10%) DMSO groups were possibly the reflection of higher live sperm rates in these groups (Figure 2, $P < 0.01$). Higher viability rates in eight and 10 percent DMSO groups supported by another study in which the post-thaw Vero cell viability was detected higher in 10% DMSO group in comparison 15% DMSO group.[15] Additionally, the live and acrosome-intact or membrane-intact sperm rates were higher in lower DMSO concentrations (Figure 3 and 4, $P < 0.05$). When total membrane-intact spermatozoon rates were similar between all the groups, the total acrosome-intact sperm rates were higher ($P < 0.05$) in 8 DMSO group than 12 DMSO group (Figure 3 and 4). Likewise, acrosome and membrane integrity rates of the monkey semen were unfavorably effected in the presence of high DMSO con-

centration.[14] Interestingly, when the results of both studies examined carefully, acrosome looks more vulnerable than the plasma membrane of the spermatozoon against high DMSO concentrations. Finally, high concentration DMSO supplementations in freezing extender dramatically decreased the both viability and motility as well as adversely affected the both acrosome and membrane integrity of the spermatozoon. In conclusion, while the addition of 8% DMSO in cryopreservation extender provided the best post-thaw semen quality, the higher DMSO concentrations (12% and 14%) detrimentally effected post thaw rabbit sperm parameters under the present experimental conditions. The presence of 8 percent DMSO in the present extender composition was found optimal to freeze rabbit semen.

Acknowledgment

The author declared that this study has received no financial support.

Kaynakça

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