Animal Reproduction Science 116 (2009) 326–334

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

The effect of trehalose on post-thaw viability and fertility of European brown hare (*Lepus europaeus* Pallas, 1778) spermatozoa

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ARTICLE INFO

Article history:
Received 25 September 2008
Received in revised form 14 January 2009
Accepted 16 February 2009
Available online 27 February 2009

Keywords:
Hare
Semen cryopreservation
DMSO
Trehalose
AI
Spermatozoa motility and viability

ABSTRACT

The aim of the study was to assess the effect of a different trehalose concentration on the post-thaw viability and fertility of European brown hare spermatozoa. The semen was collected under general anaesthesia with electroejaculation method from 4 males. Immediately after collection, the semen was diluted with an extender of the following composition: Tris 250 mM, citric acid 80 mM, glucose 70 mM, DMSO 1.0 M, egg yolk (17%, v/v), and kanamycin (80 mg/l)—Protocol I. In Protocols II and III, respectively, 50 mM and 100 mM of trehalose were added to the extender. Immediately after thawing and after 3 h incubation at 37 °C, motility characteristics of frozen/thawed semen were assessed with computer-assisted semen analysis system, and a percentage of viable, acrosome intact spermatozoa was evaluated using flow cytometry. Immediately after thawing spermatozoa motility (MOT), average path velocity (VAP), straight velocity (VSL) and curvilinear velocity (VCL) were the highest in the semen frozen without addition of trehalose ($P < 0.01$). After 3 h of incubation, MOT and spermatozoa with progressive motility (PMOT) were the lowest in the semen frozen with supplementation of 100 mM of trehalose ($P < 0.01$) and VAP, VSL, VCL and amplitude of lateral head displacement (ALH) were significantly lower in the semen frozen with supplementation of 50 mM and 100 mM of trehalose compared to the semen frozen without the addition of trehalose ($P < 0.01$), which indicates an unfavourable effect of trehalose on the motility characteristics of European brown hare spermatozoa. However, the effect of trehalose on a percentage of viable, acrosome intact spermatozoa was not observed. As a

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2009.02.012

result of artificial insemination, 54.55% females became pregnant after insemination with the semen frozen according to Protocol I, 72.73% and 50% females became pregnant after insemination with the semen supplemented with 50 mM and 100 mM of trehalose, respectively. The number of young born was 1.67 ± 0.52, 1.75 ± 1.04 and 1.60 ± 0.55, in each group, respectively. There were not any significant differences in the results of artificial insemination between groups. Summing up, it should be stated that the addition of trehalose to the extender did not have a favourable effect on post-thaw viability of European brown hare spermatozoa and an influence of trehalose on the results of artificial insemination was not found, either.

1. Introduction

Contrary to rabbits, collection of semen that would be adequate for cryopreservation from hares is difficult, since it is impossible to use artificial vagina, and semen collected with electroejaculation is often inadequate because of its small volume, as well as low motility and concentration of spermatozoa (Kozdrowski and Dubiel, 2005; Kozdrowski et al., 2006). Hares belong to those animals which are susceptible to stress and are difficult to keep in captivity. Additionally collection of blood, urine or semen samples for evaluation as well as artificial insemination (AI) require, as a rule, their premedication or anaesthesia.

In recent years a dozen or so hare breeding centres have been established in Poland with the aim of breeding and releasing hares to rebuild wild populations. The hares are kept in voliers or cages but reproductive rates in captivity are not always satisfactory. Therefore one of the methods to improve this situation is the introduction of controlled reproduction based on selection of the most valuable male hares and AI (in the cage conditions).

It was shown that after administration of GnRH (Caillol et al., 1986; Kozdrowski and Siemieniuch, 2008), hCG (Stavy and Terkel, 1992) and the combination of PMSG and hCG (Caillol et al., 1989) ovulation occurred in the hare females. Effective AI was described with the use of fresh semen (Kozdrowski and Siemieniuch, 2008), spermatozoa collected from epididymides (Stavy and Terkel, 1992) and with the frozen–thawed semen (Kozdrowski et al., 2009). It was shown that the hare semen frozen with DMSO as a cryoprotector was characterized by better post-thaw viability than the semen frozen with acetamide as a cryoprotector (Kozdrowski et al., 2009).

A lot of research that aims at the improvement of the frozen/thawed semen properties is based on the addition of nonpremating cryoprotectors such as trehalose, raffinose and methylcellulose to the extender (Dalimata and Graham, 1997; Liu et al., 1998; Yildiz et al., 2000; Aisen et al., 2000, 2002; Aboagla and Terada, 2003; Yamashiro et al., 2007; Hu et al., 2008). Some of these studies indicate a favourable effect of trehalose on the post-thaw semen viability and a favourable impact on fertility. There is not any information in the literature on the influence of trehalose on the post-thaw viability and fertility of hare spermatozoa, therefore the aim of the study was to assess the effect of a different trehalose concentrations on the post-thaw viability and fertility of the European brown hare spermatozoa.

2. Materials and methods

2.1. Males

Four adult males (body weight from 3.8 kg to 4.4 kg) were used for semen collection. During the experiment the hares were housed in individual cages, exposed to natural daylight and ambient temperature. Hares were watered and fed ad libitum on a diet composed of hay, oat, and full-component mixture for hares. Additionally, branches of fruit and willow trees were given. Semen was collected during the breeding season from the end of January to May 2008.