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Heat stress has an effect on motility and metabolic activity of rabbit spermatozoa

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ABSTRACT

In the warm months the function of the spermatozoa can be affected by the temperature of the reproductive tract of the female exposed to hyperthermic conditions. The aim of this study was to evaluate the impact of heat stress on sperm parameters in an *in vitro* model and to determine if there were seasonal effects on sperm heat tolerance. Sperm samples from 32 New Zealand White rabbits were collected in two seasons and incubated at scrotal (32.5 °C), body (37 °C) or hyperthermic (42 °C) temperatures for 3 h. Sperm viability and morphology were evaluated using nigrosin-eosin staining. Motility and metabolic activity parameters were determined using computer-assisted sperm analysis and the QBlue cell viability test, respectively. The incubation of spermatozoa at 42 °C decreased ($P < 0.05$) the mean values of total motility, curvilinear (VCL) and mean velocity (VAP) as well as the metabolic activity with respect to the incubation at 32.5 °C and 37 °C.

No seasonal effects were observed except for the highest percentages of bent and coiled tails in the cold season, and the highest mean values of VCL, linear velocity and VAP in the warm season ($P < 0.01$). The interaction between *in vitro* heat stress and season was significant for metabolic activity ($P = 0.02$). Our results suggest that rabbit spermatozoa parameters are largely modified by a short exposure to hyperthermic conditions, in terms of metabolic activity and motility parameters. Thus, a short exposure of spermatozoa to an environment of 42 °C in temperature for only 3 h may compromise sperm functionality. Additionally, sperm metabolic activity is influenced by season.

1. Introduction

Exposure of animals to high environmental temperatures has an adverse effect on physiological and reproductive functions (García-Ispierto et al., 2007; Hansen, 2009; Takahashi, 2012). High environmental temperatures during the warm months of the year or experimental exposure to heat stress (HS) can decrease conception rates, fertility and embryo development (De Rensis and Scaramuzzi, 2003; Marai et al., 2002; Yaeram et al., 2006). In several species, exposure of the testes to an acute or chronic increase of temperature during the spermatogenic cycle can reduce the sperm number in the ejaculate and affect sperm parameters, such as motility, morphology and plasma membrane integrity. These events may be fol-

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lowed by periods of partial or complete infertility (Ikeda et al., 1999; Pérez-Crespo et al., 2008; Rahman et al., 2014; Setchell, 1998; Yaeram et al., 2006).

After leaving the testis, sperm cells are vulnerable to environmental changes. The impact on sperm function can persist in the warm months of the year by the exposure of sperm cells to HS environmental conditions found in the reproductive tract of an hyperthermic female before fertilization (Hendricks et al., 2009; Hunter, 2012).

High temperatures can be achieved in the uterus of domestic species, such as cow (De Rensis and Scaramuzzi, 2003; Ealy et al., 1993; West, 2003), when the environmental temperatures are also high. Although the effects of conditions of hyperthermia have been largely analysed for the oocyte (Maya-Soriano et al., 2013, 2012), few studies have analysed the changes in sperm cell physiology when the spermatozoa reach a female genital tract under HS conditions. In this study, we wanted to compare changes in sperm cell features when they leave the testis, at a temperature of 32.5 °C (Alvarez and Storey, 1985), and they reach a genital tract at a physiological temperature (37 °C; Alvarez and Storey, 1985) or under hyperthermia conditions (42 °C; Howarth et al., 1965). Thus, this study was designed to evaluate the impact on sperm parameters of an *in vitro* HS model where rabbit sperm cells were exposed to temperatures of 32.5 °C, 37 °C or 42 °C for three hours. An additional objective was to determine if there were seasonal effects on sperm heat tolerance.

2. Material and methods

2.1. Animals and semen collection

Thirty-two New Zealand White (NZW) adult rabbit bucks (from seven to thirteen months old) from the nucleus colony at the farm of the Institut de Recerca i Tecnologia Agroalimentaries (IRTA, Torre Marimon, Spain) were used in this study. The animals belonged to the Caldes line selected for growth rate during the fattening period (Gómez et al., 2002). Each animal was housed in a single cage (85 × 40 × 30 cm) equipped with plastic footrests, a feeder and nipple drinker. Animal feed was restricted to 180 g/d of an all-mash pellet and fresh water was always available. Bucks were kept under a controlled photoperiod of 16 h of light and 8 h of darkness, and a range of temperature between 15–20 °C in winter and 20–26 °C in summer.

All males started to be trained with an artificial vagina at 4.5 month of age. A homemade polyvinyl chloride artificial vagina containing water at a temperature of 50 °C was used. One ejaculate was collected per male. Ejaculates which contained urine and calcium carbonate deposits on visual inspection were discarded. After this initial preselection, gel plugs were removed and each sperm sample was diluted 1:3 (vol/vol) in a commercial liquid diluent for rabbit semen (Galap, IVM Technologies, Saint Ouen sur Iton, France). Ejaculates were stored until evaluation in an isothermal chamber at 37 °C for no more than 30 min after collection.

2.2. Sperm preparation

Two millilitres of the diluted ejaculate were used as a non-incubated sperm control, the rest of the sample was split between three vials. For the control group, sperm cells were incubated at 32.5 °C for 3 h, an optimal temperature for testicular germ cells. For HS conditions, sperm cells were incubated at abdominal temperature (37 °C), or at hyperthermic conditions (42 °C) for 3 h. The incubation was done in a humidified air atmosphere with 5% of CO_2. After 3 h of incubation, sperm viability, morphology, motility parameters and metabolic activity were evaluated for all the experimental groups (non-incubated control, and sperm cells incubated at 32.5 °C, 37 °C or 42 °C).

2.3. Evaluation of sperm viability and morphology

The nigrosin-eosin stain method was used to evaluate viability and morphology. Smears of the sperm suspensions were prepared by mixing 10 μL of sperm sample with 10 μL of stain, smearing the mixture over a glass slide and leaving to air-dry. Then the slide was covered with mounting medium and a cover glass. The spermatozoa were evaluated by counting at least 200 cells per slide under an optical microscope (Motic BA210, Spain) at ×1000 magnification (oil immersion). Spermatozoa that remained white or unstained were considered to be viable whereas non-viable sperm cells stained pink, since the integrity of their plasma membranes had been compromised causing an increase in membrane permeability that led to uptake of the dye (Bamba, 1988). The following proportions were calculated (%): sperm viability, sperm with acrosome abnormalities, sperm with proximal and distal cytoplasmic droplets, sperm with morphological abnormalities of the head, sperm with morphological abnormalities of the tail, and sperm without tails.

2.4. Sperm motility parameters

Motility characteristics were determined using a computer-assisted sperm analysis system (CASA system, Proiser SL, Valencia, Spain). The CASA system is based on the analysis of 25 consecutive digital images taken from a single field at a magnification of 100× on a dark ground with a time lapse of 1 s.

Sample aliquots (5 μL) were placed on a pre-warmed slide and viewed in a phase contrast microscope equipped with a warm stage at 37 °C. Four to five separate fields were taken for each sample and a minimum of 200 cells/sample were analysed. The motility descriptors obtained after CASA were: Curvilinear velocity (VCL), as the mean path velocity of the sperm head along its actual trajectory (units: μm/s), Linear velocity (VSL), as the mean path velocity of the sperm head along its average from its first to its last position (units: μm/s), Mean velocity (VAP), as the mean velocity of the sperm head along its average trajectory (units: μm/s).

Finally, total motility (MT) was defined as the percentage of spermatozoa that showed a VAP above 10 μm/s, and progressive motility (MP) as the percentage of spermato-