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Retinol might stabilize sperm acrosomal membrane in situations of oxidative stress because of high temperatures

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ABSTRACT

High temperatures have negative effects on sperm quality leading to temporary or permanent sterility. The study tried to confirm the harmful effects of high temperatures on epididymal sperm cells in comparison with other temperatures (scrotal, environmental, and refrigeration temperatures), the main objective was the assessment of the addition of retinol as an antioxidant agent to improve sperm quality parameters. Testes from 10 bulls were collected from a slaughterhouse. Sperm cells were flushed from the cauda epididymis and deferent duct and assessed for sperm quality parameters at recovery. Afterward, sperm cell samples were exposed to one of four different temperatures (4 °C, 22 °C, 32 °C, and 41.5 °C for 3 hours) in presence or absence of retinol in the storage extender. Percentages of viability and morphologic abnormalities were determined using eosin-nigrosin staining. Acrosome integrity and sperm plasma membrane integrity were assessed by fluorescence *Pisum sativum agglutinin* lectin (FITC-PSA) staining and the hypo-osmotic swelling test, respectively. Total and progressive motility were analyzed by computer-assisted sperm analysis. Sperm quality parameters were mainly affected by high temperatures (41.5 °C). The addition of all-trans-retinol to the storage extender did not show any effect on sperm quality parameters. However, the percentage of sperm cells with altered acrosome was significantly reduced when retinol was present in the extender under heat stress conditions (41.5 °C). In conclusion, retinol might stabilize sperm acrosomal membrane in situations of oxidative stress because of high temperatures.

1. Introduction

The Earth system has been changing rapidly over the past several decades because of natural and anthropic factors. Studies have stated an increase in the global average surface temperature of approximately 0.6 °C over the past 20th century, with predicted increments over 0.1 °C to 0.2 °C per decade [1], with an increase in medium and maximum temperatures of 1.5 °C to 2.1 °C in 2020 [2]. These data suggest a significant increase in heat stress pressure on the global human population, animals, and plants [2], displacing life from their thermal comfort zone. In accordance, animals in our geographic area are submitted to high temperatures for 20 to 31 days during the warm months (May to September) and even 4 °C days during the cold months of the year (October to April) [3]. Heat stress is known to alter the physiology of livestock, reducing male and female reproduction and production, and increasing mortality [4].

General effects of heat stress in the male have been described as an increase in rectal temperature and respiratory rate [5–7], and negative effects on production and reproduction leading to subfertility, temporary or permanent sterility [8–11]. It has also been described a decrease in testis weight [12–14], ejaculate volume [6], sperm cell concentration, total sperm output [6,15], fertility and pregnancy rate [16,17]. Moreover, the direct effects of high temperatures reported on sperm cells are a decrease in the percentage of live and morphologically normal sperm cells

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[18–20], a loss of motility [21,22], alterations in acrosome and plasma membrane integrity [6,20,23–25], sperm chromatin stability [26,27], embryonic loss [5,28,29] and sex ratio distortion [17].

It is noteworthy that cellular exposure to heat stress increases the production of reactive oxygen species (ROS) promoting cellular oxidation events [30,31]. Sperm cell membranes composed of a high content of polyunsaturated fatty acids (PUFA) and low cholesterol levels (i.e., bull, boar, or ram) make membranes susceptible to peroxidative damage, compared with those with low PUFA and high cholesterol levels (i.e., dog, human, rabbit), making these species more resistant to cold shock [32,33]. The high concentrations of PUFA within the lipid structure require efficient antioxidant systems to defend against peroxidative damage produced by ROS [33,34]. However, the protective antioxidant systems in sperm cells are primarily of cytoplasmic origin, which is mostly discarded during the terminal stages of differentiation [35], and it is also reduced by the storage protocol [36,37]. Consequently, sperm cells are unable to resynthesize their membrane components [38], which leads to structural damage [39] and subsequent sperm dysfunction [40].

The administration of antioxidant agents *in vivo* has been shown to provide slight improvements in pregnancy rates in heat-stressed cows [41]. On the other hand, the addition of antioxidant ROS scavengers *in vitro* as supplementation of culture media has proved beneficial in oocyte/embryo culture [42–44]. Hence, antioxidant molecules could reduce the impact of oxidative stress, and thus improve semen quality [35,45–47]. There are many varieties of antioxidant agents that could be used to improve sperm quality under oxidative events, without completely eliminating ROS, because oxidative mechanisms play an important role in the physiologic control of mammalian sperm functions (sperm capacitation or sperm-egg fusion) [48,49].

The aim of the study was to assess the use of retinol as an antioxidant agent for improving sperm quality after the oxidative stress produced by heat stress submitting epididymal sperm cells from bulls to *in vitro* high temperatures simulating the conditions reported during the summer season.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Sigma (Madrid, Spain) unless otherwise indicated.

2.2. Animals and sample collection

The study was performed in North-Eastern Spain during spring (April to May), a temperate season in our location [50]. Briefly, mean temperature (T), maximal T, minimal T, mean relative humidity, minimal relative humidity, mean T-humidity index, and maximal T-humidity index for this season were 16.9 °C, 23.55 °C, 10.65 °C, 66.85%, 42.35%, 60.65 and 68.85, respectively.

Testes from 10 Friesian bulls (12 to 30 months old) with good body condition, kept in open stalls and fed *ad libitum,* were recovered from the slaughterhouse and transported to the laboratory at room temperature within 15 minutes of slaughter. When in the laboratory, testes were measured and dissected. Epididymal sperm cells were recovered after flushing cauda epididymis and vas deferens with 10 mL of Kenney medium [51] at room temperature, composed mainly of milk and glucose. At recovery, sperm quality parameters were assessed.

2.3. Experimental design

After initial sperm cell evaluation, samples were centrifuged at 2500 rpm for 10 minutes at 25 °C (Hermle Z300K) and resuspended with the storage extender. Four study groups were then established as follows: refrigeration temperature (RT; 4 °C), environmental temperature (ET; 22 °C), scrotal temperature (ST; 32 °C), and rectal temperature simulating that reached during the central part of a hot summer day (HT; 41.5 °C) [52]. On the other hand, the same four groups of study were established with 6 μM retinol (all-trans-retinol; RO; [53,54]) as an antioxidant agent added to the storage extender (RT with RO, ET with RO, ST with RO, HT with RO).

2.4. Analysis of sperm quality parameters

Sperm cell concentration and total sperm number were determined after counting in a hemocytometer chamber (Neubauer improved). Percentages of viability and morphologic abnormalities were determined using eosin-nigrosin staining. This technique shows viable sperm cells with a uniform, white stain in all of the cells, whereas the presence of a pinkish stain was indicative of nonviable sperm cells [55]. Acrosome integrity was assessed by fluorescence *Pisum sativum agglutinin* lectin (FITC-PSA) staining. Sperm plasma membrane functional integrity was assessed by the use of the hypo-osmotic swelling test (HOST) as reported by Gholami et al. [56]. All determinations were performed after analyzing a minimum of 200 sperm cells per sample using an optical microscope (magnification × 1000). Total motility and progressive motility was analyzed by computer-assisted sperm analysis (CASA; Integrated Sperm Analysis System, V1.2, Proiser S.L., Valencia, Spain). Five-microliter aliquots of prewarmed samples were placed on a warmed (37 °C) slide and covered with a 22 mm^2 coverslip. The analysis was based upon the study of 25 consecutive, digitalized photographic images obtained from a single field at magnification × 100 on a dark field. These 25 consecutive photographs were taken in a time lapse of 1 second, which implied a velocity of image-capturing of one photograph every 40 ms. Four to five separate fields were taken for each sample. The motility descriptors obtained after computer-assisted sperm analyses were: curvilinear velocity (VCL; μm/s); linear velocity (VSL; μm/s); mean velocity (VAP; μm/s); linearity coefficient (LIN; %); straightness coefficient (STR; %); wobble coefficient (WOB; %); mean amplitude of lateral head displacement (ALH; μm), and frequency of head displacement (BCF; Hz).

Finally, total motility was defined as the percentage of sperm cells which showed a VAP greater than 10 μm/s, and