Theriogenology 79 (2013) 299–305

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Bovine oocytes show a higher tolerance to heat shock in the warm compared with the cold season of the year

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ARTICLE INFO

Article history:
Received 26 November 2010
Received in revised form 25 August 2012
Accepted 27 August 2012

Keywords:
Oocyte
Heat shock
Stress tolerance
Cortical granules
Cold season
Warm season

ABSTRACT

Heat stress is especially harmful for bovine ovarian follicle development and oocyte competence. In this study, we assessed the effects of heat shock on oocyte maturation in oocytes collected during the cold (February–March; n = 114) or warm (May–June; n = 116) periods of the year. In both cases, cumulus-oocyte complexes were matured under control (38 °C) and heat shock conditions (41.5 °C, 18–21 h of maturation). For each oocyte, nuclear stage, cortical granule distribution and steroidogenic activity of cumulus cells were evaluated. Based on the odds ratio, heat-shocked oocytes were 26.83 times more likely to show an anomalous metaphase II morphology. When matured under heat shock conditions, oocytes obtained in both seasons were similarly affected in terms of nuclear maturation, whereas a seasonal effect was observed on cytoplasmic maturation. For oocytes collected during the cold season, the likelihood to show an anomalous maturation was 25.96 times higher when exposed to the heat treatment than when matured under control conditions. By contrast, oocytes collected during the warm season matured under control or heat shock did not show significant risk of showing an anomalous cytoplasmic maturation. Our findings indicate an increased rate of premature oocytes in response to heat shock as well as a higher tolerance to this stress of oocytes harvested in the warm season compared with those collected in the colder period.

1. Introduction

In dairy cows, the optimum air temperature range for milk production and reproduction is 25 °C to 26 °C [1,2]. In northeastern Spain, animals endure high temperatures (> 25 °C) for 20 to 31 days in each of the warm months (May–September) and up to 4 days in each of cold months (October–April) [3]. Nevertheless, because of high genetic selection for milk production, a decline in heat tolerance has recently been observed [4]. Heat stress is known to cause an increase in rectal temperature and reductions in dry matter intake, milk production, and fertility [2,5,6]. Problems related to heat stress have economic repercussions worldwide but especially affect animals in the Northern hemisphere [4,7]. Conception rates in these animals are lower in summer (June–September) than winter (10%–20% vs. 40%–60%) [4,8,9].

Fertility effects of heat stress are the outcome of damage to antral follicles produced in warm months [10]. Thus, after exposure to high temperatures, three to four estrous cycles are needed to recover fertility [11,12]. Heat stress to oocytes is especially harmful 3 days before and 1 day after insemination, and affects follicle development and oocyte competence [1,13]. Oocyte maturation is usually assessed in terms of nuclear and cytoplasmic events [14–16], which are usually highly coordinated [17]. In most of the species examined so far, nuclear maturation involves the transition from a germinal vesicle nucleus to a second metaphase arrangement of the chromosomes and formation of a first polar body by the time of ovulation. Cytoplasmic maturation

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0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.08.020

manifests as changes in protein contents, but most conspicuously in the redistribution of organelles such as cortical granules (CG) [18,19]. During resumption of meiosis, the CG migrates from the Golgi apparatus to the vitelline surface, assuming a position 0.4 to 0.6 μm below the plasma membrane [20]. Only when situated just beneath the plasma membrane can they undergo exocytosis by fusing with the egg membrane. This fusion enables the release of CG contents into the perivitelline space and is an important step in membrane maturation and blockade of polyspermy [19,21,22].

Under high temperature conditions, anomalous chromosome segregation and cytoplasmic defects in oocyte maturation have been attributed to a rise in reactive oxygen species [12,23,24]. Thus, oocytes matured under heat conditions show lower rates of nuclear maturation, chromatin fragmentation, and premature translocation of cortical granules [25–27]. A recent study has shown that heat shock causes premature aging of bovine oocytes [16]. Notwithstanding, Ealy et al. [5] and Hansen and Arechiga [28] found that by preincubating oocytes and embryos at slightly higher temperatures than usual, heat adaptation occurred before heat shock could block cell apoptosis.

Heat stress has been linked to reduced fertility in many countries [8,29], producing high economic losses. In particular, reproductive problems are observed when high temperatures coincide with the time of insemination [13,30]. This study was designed to examine the effects of heat shock on oocyte maturation and to identify possible seasonal differences in oocyte heat tolerance.

2. Materials and methods

2.1. Experimental design

In northern Spain, we can clearly differentiate a warmer (May–September) and colder (October–April) period of the year [3]. Mean monthly climate variables were similar to those published previously for the area of the study [31]. Briefly, mean temperature (T), maximal T, minimal T, mean relative humidity, minimal relative humidity, mean T-humidity index (THI), and maximal THI were 10.3 °C, 16.6 °C, 4.4 °C, 71%, 43%, 50.2 THI, and 60.3 THI for the cold period of the study (February–March) and 20.6 °C, 26.9 °C, 14.3 °C, 65%, 37%, and 65.6 THI, and 72.9 THI, respectively, for the warm period of the study (May–June). Seasonal effects were thus evaluated in bovine oocytes ($n = 230$) collected in the cold season (CS; $n = 114$) or warm season (WS; $n = 116$). Two study groups were established as follows: control oocytes (C) cultured at 38.5 °C for 22 hours; and heat-shocked (HS) oocytes subjected 3 hours at the end of the maturation period (from 18 to 21 h) to 41.5 °C, simulating the animal rectal temperature during the warmer hours of the day [26]. The four groups finally compared were: CCS; HSCS; CWS; and HSWS.

2.2. Chemicals and reagents

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

2.3. Collection of oocytes

Ovaries from heifers recovered at a slaughterhouse were placed in Dulbecco's phosphate buffered saline solution (PBS) supplemented with 1% (vol/vol) antibiotic/antimycotic solution (AA; 10,000 U penicillin, 10 mg streptomycin, and 25 mg amphotericin B per mL) and transported to the laboratory at room temperature. The mesovarium, oviduct, and fat were removed, and the ovaries then washed twice in warm sterile PBS and kept at 37.5 °C until follicle puncture within 2 h of ovary recovery. Ovarian follicles (2–8 mm) were aspirated using an 18-ga needle and 5 mL syringe and placed in working medium (TCM 199-HEPES and 1% vol/vol AA solution).

2.4. In vitro maturation and oocyte preparation for staining

For *in vitro* maturation (IVM), cumulus-oocyte complexes (COCs) larger than 120 μm with three or more layers of compact cumulus cells and a homogeneous translucent ooplasm were selected.

Selected COCs were washed twice in working medium and randomly placed in groups of 20 to 25 in four-well dishes (Nunc 150288; Biocen, Barcelona, Spain) containing 500 μL of maturation medium (TCM 199 supplemented with 20 μg/mL epidermal growth factor, 0.2 mM sodium pyruvate, and 1% vol/vol AA solution).

All procedures took place in a laminar flow cabinet within 2 h of follicular puncture.

COCs were cultured according to the experimental design in an atmosphere of 5% (vol/vol) CO_2 in humidified air. Control and HS oocytes were incubated in two different CO_2 incubators equipped with temperature and humidity probes.

Cumulus-oocyte complexes were morphologically assessed for cumulus cell expansion after 22 hours of IVM. Next, the COCs were denuded of cumulus cells by pipetting inside the wells and washing three times in PBS with 0.05% (wt/vol) bovine serum albumin (BSA; Fraction V).

Maturation media were kept in Eppendorf tubes and recovered after centrifugation (2500 rpm, 37 °C for 15 minutes) for hormone determinations. Aliquots were kept at −20 °C until analysis.

Denuded oocytes were immersed in PBS containing 0.4% (wt/vol) Pronase E at 37.5 °C for 1 to 2 min until zona pellucida digestion. The oocytes were then washed five times in PBS-BSA 0.05% (wt/vol) and fixed in a PBS solution containing 4% (wt/vol) paraformaldehyde (Panreac, PRS 141451.1210) at refrigeration temperature for 30 min.

Oocytes were washed three times in PBS-BSA 0.05% and then incubated for 5 min in a permeabilizing solution of PBS containing 0.3% Triton × 100 and 0.05% (wt/vol) BSA at room temperature. Next, the oocytes were washed five times in PBS-BSA 0.05%.

2.5. Cortical granule staining

Oocytes were incubated in dark conditions for 30 min at room temperature in a 100 μg/mL fluorescein isothiocyanate-labeled lens culinaris agglutinin (FITC-LCA) solution and washed five times to eliminate lectin excess.