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Meiotic competence and DNA damage of porcine oocytes exposed to an elevated temperature

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Abstract

The present study was conducted to investigate the effects of the length of exposure to an elevated temperature (41 °C) on the meiotic competence and DNA damage of porcine oocytes. Oocytes were recovered from ovaries, loaded into straws, and then exposed at 41.0 or 38.5 °C (sham control) for 0, 0.5, 1.0, or 1.5 h, followed by culture for 44 h. The proportion of oocytes reaching the metaphase II (MII) stage gradually decreased with increasing exposure time, irrespective of the exposure temperature. A higher proportion of oocytes stored at 38.5 °C reached MII (57–63%) than those exposed to 41 °C (14–29%; $P < 0.01$). The proportion of total oocytes with DNA fragmentation gradually increased with increasing exposure time, irrespective of the exposure temperature. The proportion of DNA fragmentation in total oocytes exposed to 41 °C (37–57%) was higher ($P < 0.01$) than that in total oocytes stored at 38.5 °C (14–24%). When the oocytes were stored at 38.5 °C for up to 1.5 h, there were no differences in the proportions of MII-stage oocytes, with DNA-fragmented nuclei among all groups ($P > 0.05$). However, a higher proportion of MII-stage oocytes exposed to 41 °C for more than 1 h exhibited DNA-fragmented nuclei, compared with MII-stage oocytes stored at 38.5 °C ($P < 0.05$). In conclusion, exposure of porcine oocytes to an elevated temperature had a detrimental effect on the meiotic competence and quality of oocytes; furthermore, the effect was dependent on the duration of exposure.

Keywords: Heat stress; Storage; Apoptosis; Oocyte maturation; Pig

1. Introduction

In vitro-produced mammalian embryos are usually derived from oocytes collected from ovaries obtained at an abbatoir. In general, the ovaries are preserved in physiological saline at approximately 30–35 °C and transported to the laboratory. During transportation of ovaries and manipulation of oocytes, the oocytes (within or outside ovarian follicles) are often exposed to a wide range of temperatures that may ultimately affect subsequent development. Cooling of mammalian oocytes to sub-physiological temperatures is well known to affect their viability through the induction of various abnormalities at all stages of meiosis [1–4]. In particular, porcine oocytes at the germinal vesicle (GV) stage were highly sensitivity to chilling [5]. In a previous study, we also confirmed that the meiotic competence of porcine oocytes from ovaries stored at less than 25 °C decreased [6]. However, little informa-

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tion is available concerning the effects of elevated temperature during the manipulation of oocytes on oocyte quality and meiotic competence.

It is noteworthy that elevated temperature is a major factor responsible for the reduced fertility in farm animals during the hot season in tropical areas. Heat stress (HS) can compromise reproductive events by decreasing the expression of estrous behavior, altering ovarian follicular development, compromising oocyte competence, and inhibiting embryonic development [7,8]. Based on field fertility data, conception rates in cattle were significantly lower during the hot summer compared with the cool season [9]. Moreover, embryo viability in cows was lower in the hot season than in the cool season [10]. In most mammalian species, the deleterious effects of HS on embryonic mortality were most pronounced at or near the time of estrous, relative to the rest of the estrous cycle [11–13].

Direct exposure of bovine oocytes at the GV-stage to an elevated temperature (41 °C) for 12 h reduced their ability to complete nuclear maturation and development after fertilization [14]. Moreover, some studies have demonstrated DNA fragmentation and cytoskeleton disruption of oocytes after direct exposure to elevated temperature before or during maturation culture [15,16]. Roth and Hansen [15] reported that elevated temperatures within the physiological range (40–41 °C) during maturation culture increased programmed cell death in bovine oocytes. These observations suggested that the exposure of oocytes to elevated temperature induced DNA damage in oocytes prior to fertilization.

The objective of this study was to investigate the effects of the length of exposure to an elevated temperature (41 °C) on the meiotic competence and DNA damage of GV-stage porcine oocytes.

2. Materials and methods

2.1. Exposure to an elevated temperature and in vitro maturation (IVM) of oocytes

Ovaries from prepubertal crossbred gilts, approximately 6 months old, were collected at a local abbatoir and transported to the laboratory (within 3 h) in physiological saline [0.85% (w/v) NaCl] at 35 °C. The cortex of each ovary was sliced repeatedly with a scalpel blade to release cumulus–oocyte complexes (COCs) from antral follicles in a 90-mm culture dish containing modified-PBS (mPBS; Embryotech, Nihon Zenyaku Kogyo, Fukushima, Japan). The COCs with at least two dense layers of cumulus cells were collected and washed twice with mPBS. To assess the effects of exposure of oocytes to elevated temperature on oocyte meiotic competence and nuclear damage, the COCs were randomly assigned to seven groups. The COCs were transferred into fresh mPBS and then loaded into 0.25-mL French plastic straws (I.M.V., L'Aigle, France), with 7 or 10 COCs per straw. The plastic straws were loaded as follows: a 5 cm length of the straw was filled with mPBS, followed by a 0.5 cm air bubble, approximately 1.0 cm of mPBS containing the COCs, another air bubble, the mPBS (approximately 3 cm) and a further air bubble. The straws were stored at 41 °C in an incubator for 0.5, 1.0, or 1.5 h. In order to evaluate the exposure conditions (sham control), some straws containing the COCs were stored at 38.5 °C for 0, 0.5, 1.0, or 1.5 h. After each exposure time, the contents of the straws were drained into polystyrene culture dishes. The COCs were immediately transferred into a maturation medium, a modified North Carolina State University (NCSU)-37 solution [17] supplemented with 0.6 mM cysteine, 1 mM dibutyryl cyclic AMP (dbcAMP; Sigma, St. Louis, MO, USA), 10 IU/mL equine chorionic gonadotropin (eCG; KawasakiMitaka K.K., Tokyo, Japan), 10 IU/mL human chorionic gonadotropin (hCG; KawasakiMitaka K.K.), 50 μg/mL gentamicin (Sigma) and 10% (v/v) porcine follicular fluid. Approximately 10 COCs were cultured (for 22 h) in each 100 μL drop of the maturation medium, covered with a layer of mineral oil (Sigma) in a 35 mm × 10 mm Petri dish. They were then transferred to the maturation medium without hormones and dbcAMP and cultured for an additional 22 h. All cultures were performed in a 38.5 °C humidified incubator containing 5% CO_2 in air.

2.2. Analysis of meiotic stage and DNA damage of oocytes

After maturation culture, the meiotic stage and DNA damage of oocytes were analyzed using a combined technique for simultaneous nuclear staining and the terminal deoxynucleotidyl transferase (TdT) nick-end labeling (TUNEL), by a modification of the procedures previously described by Otoi et al. [18]. Briefly, oocytes were mechanically denuded from cumulus cells in PBS (Invitrogen, Carlsbad, CA, USA), supplemented with 1 mg/mL hyaluronidase (Sigma). Denuded oocytes were washed four times in PBS containing 3 mg/mL polyvinylalcohol (PBS–PVA) and fixed overnight at 4 °C in 3.7% (w/v) paraformaldehyde diluted in PBS. After fixation, the oocytes were washed four times in PBS–PVA, permeabilized in PBS containing 0.1% (v/v) Triton-X100 for 1 h, and incubated in a PBS containing