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Original Research Article

Astaxanthin present in the maturation medium reduces negative effects of heat shock on the developmental competence of porcine oocytes

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ABSTRACT

Astaxanthin, one of the most common carotenoids, elicits antioxidant effects on cellular viability and embryonic development. This study was conducted to investigate the effects of astaxanthin on maturation, fertilization and development of porcine oocytes matured *in vitro* under heat stress conditions, and then fertilized and cultured under standard conditions. Porcine oocytes were cultured in maturation medium supplemented with different concentrations of astaxanthin (0, 0.25, 0.5 or 1 ppm) for 46 h at either 38.5 or 41 °C. In comparison to oocytes cultured at 38.5 °C, the exposure of porcine oocytes to 41.0 °C during *in vitro* maturation (IVM) significantly inhibited maturation and development of fertilized oocytes to the blastocyst stage. Supplementation of maturation medium with astaxanthin (0.5 ppm) significantly improved oocyte maturation, fertilization and development to the blastocysts stage in both oocyte groups. However, the total cell number and apoptosis index of blastocysts did not differ among groups. Moreover, astaxanthin (0.5 ppm) significantly increased the rate of oocytes that reached metaphase II and decreased proportion of apoptotic oocytes exposed to H_2O_2 (1.0 mM) during IVM. In summary, we demonstrated that supplementation of maturation medium with astaxanthin (0.5 ppm) exerted antioxidative effects and improved the ability of maturation, fertilization, and development of porcine oocytes exposed to heat stress.

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1. Introduction

Reproductive performance in livestock is generally affected by climatic conditions [1,2]. Heat stress is a crucial factor that can disrupt reproductive processes by altering the regulation of body temperature [3]. Adverse climatic conditions, such as elevated temperature and heat indices have been shown to elicit significant effects on litter size, farrowing rate and weaning to first service interval in Yorkshire and Landrace sows [4]. Heat stress has been demonstrated to significantly reduce conception rates in gilts exposed to higher ambient temperature during the early period of implantation [5]. Moreover, compared with other species, porcine oocytes exhibited an increased sensitivity not only to low but also to elevated temperatures [6].

Roth et al. [7] suggested that activation of apoptotic processes mediated by group II of caspases, which is caused by heat stress during bovine oocyte maturation, is a critical mechanism responsible for the disruption of oocyte capacity to support early embryonic development. It has been suggested that heat stress reduces intracellular concentration of the antioxidant glutathione in embryos and that the addition of various antioxidants to culture media, including taurine, glutathione, and vitamin E, provide some thermoprotection of embryos [8]. Moreover, dietary supplementation with antioxidants such as vitamins C and E protected oocytes against aged-associated disturbances in segregation of chromosomes during maturation [9].

Astaxanthin is a common carotenoid that is extracted from fishery products [10]. Recent studies have reported that astaxanthin exhibits the ability to scavenge not only most reactive oxygen species (ROS) but also the hydroxyl radical [11]. As a scavenger of peroxyl radicals, astaxanthin is twice as effective as β-carotene in liposomes [12] and exceeds in quenching of reactive oxygen in *E. coli* compared with β-carotene and vitamin E [13]. It has been demonstrated that astaxanthin improved the development of *in vitro* bovine embryos exposed to heat stress by reducing the expression of stress-inducible genes [14]. However, limited information concerning the effects of astaxanthin on oocyte *in vitro* maturation and early embryonic development is currently available for pigs.

We hypothesized that astaxanthin supplementation during *in vitro* maturation protects porcine oocytes from the deleterious effects of heat stress and improves the development of oocytes after maturation culture. Therefore, in this study, we investigated the effects of astaxanthin supplementation during *in vitro* maturation (IVM) on the ability of maturation, fertilization, and further embryonic development of porcine oocytes exposed to 41.0 °C. Hydrogen peroxide has been used to induce oxidative stress because its cellular effects and chemical reactivity have been well studied. Therefore, we evaluated antioxidant effects of the astaxanthin treatment on the meiotic competence and apoptosis of porcine oocytes exposed to hydrogen peroxide during IVM.

2. Materials and methods

2.1. In vitro *maturation and assessment of meiotic status*

Porcine ovaries were obtained from 6- to 7-month-old peripubertal crossbred gilts (Landrace × Large White) at a local slaughterhouse and transported to the laboratory within 3 h in physiological saline (0.9% (w/v) NaCl) at 30 °C. Ovaries were washed three times with the modified phosphate-buffered saline (m-PBS; Nihonzenyaku, Fukushima, Japan) supplemented with 100 IU/mL penicillin G potassium (Meiji, Tokyo, Japan) and 0.1 mg/mL streptomycin sulfate (Meiji). The cumulus–oocyte complexes (COCs) were collected from follicles by slicing the ovarian surface using a surgical blade (about 15 COCs per ovary). Only COCs with uniformly dark-pigmented ooplasm and intact cumulus cell masses were collected. Approximately 50 COCs were cultured in 500 μL maturation medium, consisting of 25 mM HEPES tissue culture medium 199 with Earle's salts (TCM 199; Invitrogen Co., Carlsbad,

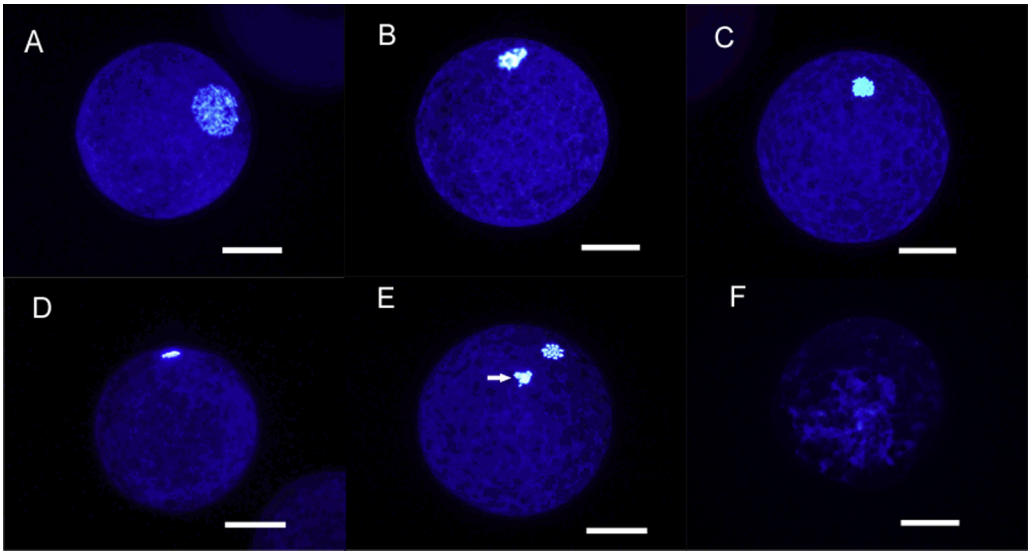

Fig. 1 – Representative images of developmental stage of porcine oocytes stained by Hoechst 33342. (A) Germinal vesicle, (B) germinal vesicle break down, (C) metaphase I, (D) anaphase I, (E) metaphase II with a polar body (arrow) and (F) degenerated oocyte. Scale bar = 50 μm.