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Effects of culture media and energy sources on the inhibition of nuclear maturation in bovine oocytes

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Abstract

The influence of the culture medium and energy sources on spontaneous nuclear maturation and inhibition of maturation in bovine cumulus-enclosed oocytes (CEO) was examined. CEO were cultured in Medium 199, minimum essential medium, M16, or synthetic oviduct fluid (SOF), all containing 3 mg/mL bovine serum albumin (BSA), and SOF without BSA, alone or supplemented with hypoxanthine (HYPO, 4 mM) or forskolin (FSK, 100 μM) for 21 h. More CEO remained at the GV stage in M16 compared to other media ($P < 0.05$). Supplementation with HYPO increased and FSK reduced the percentage of CEO remaining at the GV stage ($P < 0.05$) only in M16. The effects of energy sources, in the absence or presence of HYPO or FSK, were examined in CEO cultured in M16 salts + PVA. Glucose (0.5 and 5.5 mM), pyruvate (0.32 and 3.2 mM), lactate (3.3 mM) and glutamine (1.3 mM) significantly reduced the percentage of CEO remaining at the GV stage compared to M16 salts alone; only glutamine significantly increased the percentage of CEO at the MII stage compared to M16 salts. In M16 salts + HYPO, glucose (0.5 mM), pyruvate (0.32 mM), lactate (3.3 mM) and glutamine (1.3 mM) significantly reduced the percentage of GV and degenerate oocytes and increased the percentage of CEO at the MI stage. In M16 salts + FSK, the energy sources significantly decreased the percentage of oocytes with condensed chromosomes and increased the percentage of CEO reaching metaphase I. In conclusion, meiotic inhibitors had different effects in different culture media and glucose, pyruvate, lactate and glutamine were stimulatory to nuclear maturation. It was noteworthy that some of the results obtained were contrary to previous findings in mouse oocytes.

Keywords: Culture medium; Bovine oocytes; Meiosis; Energy sources

1. Introduction

In mammalian oocytes, meiosis is initiated during fetal life and is subsequently arrested at the diplotene stage of the first meiotic division until ovulation. The completion of the first meiotic division is triggered by the preovulatory hormonal surge; meiosis progresses to the metaphase II stage, where it is again arrested until fertilization. However, when meiotically competent mammalian oocytes are removed from follicles and cultured, they spontaneously resume meiotic maturation without hormonal stimulation.

The mechanisms involved in the control of meiotic arrest and induction are not fully understood. However, for mouse oocytes, media composition greatly affected nuclear maturation in vitro [1]. For example, the presence of specific energy sources, their combinations, concentrations and the background medium influenced the rate of spontaneous nuclear maturation, the efficiency of hypoxanthine and dibutyryl cAMP in maintaining meiotic arrest and the efficiency of FSH in stimulating nuclear maturation in arrested oocytes [2–4].

The energy requirements for bovine oocyte nuclear maturation are not as well defined as in the mouse. Lim

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et al. [5] showed that without energy substrates, proteins or hormones, bovine cumulus-enclosed oocytes (CEO) resumed meiosis, but failed to reach metaphase II. Pyruvate and lactate stimulated nuclear maturation of bovine CEO to MII, but they were not as effective as glucose [5]. Pyruvate, however, is important for nuclear maturation of denuded oocytes (DO), in which glucose metabolism is very low [6,7]. Hashimoto et al. [8] reported different effects of glucose and pyruvate, whether oocytes were cultured at 20 or 5% O_2, suggesting that, as in the mouse, the effects of energy substrates on bovine oocyte nuclear maturation may depend on culture conditions. Glutamine is metabolized by bovine cumulus–oocyte complexes (COC) and DO, but optimal metabolism requires both compartments [7,9]. Although glutamine was beneficial for acquisition of developmental competence in bovine oocytes matured in vitro [10] there is little information regarding its effect on bovine oocyte nuclear maturation. Clearly, more work is needed to determine the effects of energy sources on bovine oocyte nuclear maturation.

In domestic species, it would be desirable to delay spontaneous nuclear maturation in vitro in order to increase developmental competence of oocytes once maturation is allowed to resume [11]. However, some of the molecules that inhibit nuclear maturation in mouse oocytes were not as effective in bovine oocytes. For example, hypoxanthine and cAMP inhibited nuclear maturation in bovine oocytes, but the inhibition is transient and higher concentrations were required [12]. In general, experiments to study the control of meiosis or the acquisition of developmental competence in bovine oocytes are performed in Medium 199 containing serum [13,14]; however, defined media such as synthetic oviduct fluid [15] are being increasingly used [16,17]. Perhaps altering media composition would allow more efficient inhibition of bovine oocyte nuclear maturation by certain molecules. There is little work published on the effects of medium composition on the ability of inhibitors to maintain meiotic arrest in bovine oocytes. Therefore, the objective of the present study was to examine the influence of the culture medium and energy sources on spontaneous nuclear maturation, and inhibition of maturation by hypoxanthine and forskolin, in bovine cumulus-enclosed oocytes in vitro.

2. Materials and methods

2.1. Oocyte recovery and culture

Bovine ovaries from cows and heifers were collected at an abattoir about 15 min after slaughter. Cumulus-enclosed oocytes (CEO) were recovered from the ovaries by aspiration of follicles. Oocytes with an unexpanded mass of cumulus cells and homogeneous cytoplasm were recovered under a stereomicroscope. The CEO were washed once in Medium 199-Hepes-PVA (Medium 199 with Earle's salts and L-glutamine (Sigma–Aldrich Canada, Oakville, Ont., Canada), supplemented with 20 mM Hepes, 3 mg/mL polyvinyl alcohol (PVA), and 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA) and twice in the appropriate culture medium. For all experiments, CEO were cultured at 39 °C in a moisture-saturated atmosphere of 5% CO_2 in air for 21 h in groups of 10–20 in 500 μL of culture medium.

2.2. Fixation of oocytes and evaluation of nuclear maturation

At the end of the culture period, CEO were transferred to 1.5-mL tubes containing 400 μL of trypsin solution (Gibco; 2.5 mg/mL in phosphate-buffered saline) and vortex-agitated for 1 min to remove cumulus cells. The oocytes were then recovered under a stereomicroscope and transferred to glass slides. A mixture of vaseline and paraffin was used to maintain a coverslip in contact with the oocytes. Coverslips were secured with epoxy glue and the slides immersed in fixative (ethanol:acetic acid, 3:1, v/v) for at least 24 h, stained with 1% aceto-orcein and examined at 320× magnification. Oocytes were classified as being at one of the following stages: germinal vesicle stage (GV), germinal vesicle breakdown (GVBD), condensed chromosomes (Cond), metaphase I (MI), anaphase I, telophase I or metaphase II (MII). Oocytes with no visible or abnormal chromatin configuration were classified as degenerate.

2.3. Osmolality measurements

Osmolality was measured in 10 μL aliquots of medium (with BSA or PVA and antibiotics) with a Wescor 5500 vapor pressure osmometer (Logan, UT, USA). Osmolality is expressed as the mean of at least three readings of three different medium samples.

2.4. Experimental design

2.4.1. Effects of different culture media without or with hypoxanthine or forskolin on nuclear maturation in bovine cumulus-enclosed oocytes

The media tested were: Medium 199 (M199, with Earle's salts and L-glutamine, Sigma–Aldrich Canada),