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Effects of caffeine, cumulus cell removal and aging on polyspermy and embryo development on in vitro matured and fertilized ovine oocytes

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Abstract

The objectives of these studies were to determine the effects of cumulus cell removal and caffeine treatment on the development of in vitro matured ovine oocytes aged in vitro until until fertilization. Oocytes were denuded (DO) at 24 h post-onset of maturation (hpm), control cumulus oocyte complexes (COC's) and DO groups were fertilized at 24 hpm or returned to culture in the presence or absence of 10 mM caffeine and fertilized at 30 hpm. Removal of cumulus cells and aging both increased polyspermy, caffeine reduced this increase, however, with the exception of DO's (30 hpm) vs. COC's (24 hpm) the differences were not statistically significant. Aging significantly decreased cleavage between COC groups at 24 hpm and 30 hpm and caffeine did not affect this (68.4%, 73.4%, 74.0% respectively). In contrast, the frequency of cleavage was significantly reduced in the DO (24 hpm) group as compared to COC controls (45.6% vs. 68.4% ($P < 0.05$)), however, cleavage increased in the DO group on aging (73.4%) and this was not affected by caffeine (73.0%). The percentage of COC's and DO's developing to the blastocyst stage significantly decreased on aging, caffeine treatment of DO's prevented this (31.3%, 12.7% and 29.4% respectively ($P < 0.05$)) but had no effect on COC's (4.2% vs. 3.9%). Total cell numbers in blastocysts were not statistically different (92.4 ± 5.2, 84.7 ± 3.7 and 80.4 ± 5.8 ($P > 0.05$)). In summary caffeine treatment of aged COC's had no significant effect on the frequency of development, however, in aged DO's caffeine treatment statistically increased development to blastocyst and lowered the frequency of polyspermy.

Keywords: Ovine; Oocyte; Embryo; Caffeine; Polyspermy; MPF; MAPK

1. Introduction

In the majority of mammals, oocytes are ovulated at metaphase of the second meiotic division (MII). The ovulated, matured oocyte or unfertilized egg then remains at MII until fertilization occurs and development begins. Alternatively, oocytes can be activated artificially by a range of physical or chemical stimuli including electric shock, ethanol, Ca^{2+} ionophore, or Sr^{2+}, these treatments can be applied individually or in combination with the protein synthesis inhibitor cycloheximide or the serine

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threonine kinase inhibitor di-methyl-amino-purine (DMAP) [1]. The maturation of oocytes from the germinal vesicle (GV) stage to MII is a dynamic process that requires coordination of both nuclear and cytoplasmic processes [2]. The control of nuclear maturation is intrinsically linked to the levels of two cytoplasmic protein kinases, maturation promoting factor (MPF) and mitogen activated protein kinase (MAPK) [3]. MPF is a cyclin dependent serine/threonine protein kinase, its activation occurs in late G_2 by de-phosphorylation of T14 and Y15 by cdc25 phosphatase [4]. Active MPF phosphorylates a range of proteins initiating entry into M-phase resulting in nuclear envelope breakdown, chromatin condensation and microtubular reorganization [5–7]. MAPKs are serine/threonine kinases that require phosphorylation on threonine and tyrosine residues to become activated [8,9], this involves a cascade of upstream kinases [9]. The increases in the activities of both kinases are responsible for the onset of germinal vesicle breakdown (GVBD) and required for the arrest of oocytes at metaphase of second meiotic division (MII) [10–14], MII arrest is then maintained by continued high activities of both kinases [12,14,15].

The matured (MII) oocyte acquires fertilization competence, however, the lifespan window for fertilization varies between different species [16]. If oocytes are not fertilized during this optimal time frame, then they consequently age. Aging is associated with a range of changes including; alteration of intracellular Ca^{2+} dynamics [17], decreases in the activities of both MPF and MAP kinases [18], an increase in activation sensitivity [19], alteration of cortical granule release and increased risk of polyspermy [20]. In addition a deterioration of the spindle can result in the loss of attachment of kinetochores to the spindle fibres and displacement of the chromosomes from the spindle equator [21–23]. Furthermore, an increased frequency of fragmentation, with decreased frequencies of cleavage and development to the blastocyst stage have been reported in a variety of species [16,20,24,25].

MPF activity is controlled by association of cdc2 with cyclin B and phosphorylation of cdc2 at T14 and Y15. Caffeine, a phosphodiesterase inhibitor has been reported to artificially increase the activity of MPF by inducing the dephosphorylation of cdc2 at T14 and Y15 in pig oocytes [26,27], cultured mammalian cells [28] and *Xenopus* oocytes [29]. However, it cannot restore loss of MPF activity caused by degradation of cyclin B, which occurs on aging in pig oocytes [26]. We have previously reported that treatment of in vitro matured ovine oocytes with caffeine increases activities of both MPF and MAPK and prevents the decline in kinase activities associated with aging. Furthermore, maintaining the levels of both kinases in aging oocytes prevented the acquisition of activation sensitivity [30]. In addition, caffeine treated ovine oocytes used for nuclear transfer resulted in an increased occurrence of nuclear envelope breakdown (NEBD) in the transferred nuclei, and in blastocysts with a significantly higher cell number than control groups, however, there was no improvement in the frequency of development to the blastocyst stage [31]. In this manuscript, the effects of caffeine on the incidence of polyspermy, frequency of embryo cleavage and development to blastocyst of ovine oocytes aged and then fertilized in vitro are reported and discussed.

2. Materials and methods

All chemicals and reagents were purchased from Sigma–Aldrich, Dorset, UK, unless otherwise stated.

2.1. Collection of oocytes

Ovine ovaries were collected from a local slaughter house in warm (25 °C) phosphate buffered saline (PBS), transferred to the laboratory and processed within a maximum of 2 h after collection. In the laboratory, cumulus oocyte complexes (COC's) were aspirated from follicles of 2–10 mm in diameter using a 10 mL syringe fitted with an 18-gauge needle. COC's with a uniform cytoplasm and at least 3 layers of unexpanded cumulus cells were selected for subsequent maturation.

2.2. In vitro oocyte maturation

Selected oocytes were washed three times in dissection medium (Medium 199 containing 10% FCS (Gibco Life Technologies Inc., Paisley, UK), and 1 M HEPES), and then once in maturation medium (Medium 199 containing 10% FCS (Gibco Life Technologies Inc., Paisley, UK), 5 μg/mL FSH (Vetropharm, Ireland), 5 μg/mL LH (Vetropharm, Ireland), 1 μg/mL oestradiol-17β and 50 μg/mL gentamicin). For maturation, groups of 40–45 oocytes were cultured in 500 μL of maturation medium overlaid with mineral oil in 4-well dishes (Nunclon, Rosklide, Denmark) and incubated at 39 °C in a humidified atmosphere of 5% CO_2 for a period of 24 or 30 h depending on the experimental group.