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Replacement of serum with sericin in *in vitro* maturation and culture media: Effects on embryonic developmental competence of Sanjabi sheep embryo during breeding season

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ABSTRACT

Sericin is a water-soluble component of silk and has been used as a biomaterial due to its antibacterial and ultraviolet radiation-resistant properties. This study was designed to evaluate the effect of sericin supplementation, as a serum replacement, in maturation and culture media on the meiotic competence of oocytes or *in vitro* culture of ovine embryos. In experiment 1, oocytes were matured in the presence of 10% fetal ovine serum (FOS), 0.1% polyvinyl alcohol (PVA) and different concentrations of sericin (0.1, 0.5, 1 and 2.5%), for 24 h. The addition of 0.5% sericin to maturation medium increased the rates of maturation to metaphase II of oocytes compared with those in cultures with 0.1% PVA. Following fertilization, blastocyst development was higher for oocytes matured with 0.5% of sericin compared with 0.1% PVA. However, the rates of nuclear maturation of oocytes and blastocyst development under FOS and 0.5% sericin were not significantly different. In experiment 2, presumptive zygotes were cultured in the presence of 10% FOS, 0.1% PVA and different concentrations of sericin (0.1, 0.5, 1 and 2.5%), for 7–8 days. The addition of 0.5% sericin to culture medium increased the blastocyst rate compared with those in cultures without sericin or addition of 0.1% PVA and 10% FOS. These results indicate the feasibility of sericin as an alternative protein supplement for IVM and IVC in ovine oocytes and zygotes.

1. Introduction

Various types of basic culture media that have been used for *in vitro* embryo production (IVEP) of ovine embryos, are frequently supplemented with serum such as bovine serum albumine (BSA), fetal bovine serum (FBS), or fetal ovine serum (FOS) to provide growth factors, hormones, amino acids, and proteins, which are beneficial to embryonic development [1]. Because serum are prepared and purified from blood products, they present a high risk of contamination by pathogens, viruses and prions [2]. Thus, alternative supplements have been evaluated to replace serum during IVEP in order to avoid cross contamination. The most commonly defined alternative supplements used for IVEP are synthetic macromolecules, such as polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) [2]. However, the results are controversial, and the quantity and quality of the embryos produced are often unsatisfactory.

Sericin is a sticky protein derived from the silkworm *(Bombyx mori)* cocoon. It is the second major protein component (besides fibroin) of silk and is extracted from the cocoon by a degumming process. Sericin consists of 18 kinds of amino acids most of which have strong polar side groups such as hydroxyl, carboxyl, and amino groups [3]. It is rich in aspartic acid as well as serine [4], which has a high content of the hydroxyl group. Sericin has a number of interesting properties that are the subject of contemporary research, such as the promotion of cell viability, collagen production [5], the acceleration of cell proliferation [6], the suppression of skin tumorigenesis [7], and colon carcinogenesis [8], at certain sericin concentrations in murine models. Isobe et al. [9] showed that, the addition of sericin to *in vitro* culture medium improved preimplantation development and quality of bovine embryos cultured individually by preventing oxidative stress. Furthermore, Yasmin et al. [10] indicate that supplementation with 0.1% sericin during maturation culture may improve the nuclear maturation and fertilisability of sheep oocytes. Hence, our previous results suggest that addition of 0.5% sericin during IVM, IVC or

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during both IVM and IVC may improve the meiotic competence of oocytes and the preimplantation development of ovine embryos produced *in vitro* [11–13].

Serum that includes BSA or FOS is routinely added to the culture medium for the *in vitro* maturation of sheep oocytes [14]. Along with risk of contamination by pathogens, viruses and bovine spongiform encephalopathy (BSE), and prions etc, variation between batches of added serum to the culture mediumcan result in variable IVEP results [15]. For these reasons, there has been a trend to use more defined or at least semidefined maturation media instead of undefined natural serum preparations such as FOS and FBS [10,16]. Also, to protect oocytes and embryos from oxidative stress during IVM and IVC, antioxidants can be added to serum-free media. It has been shown that sericin has a strong antioxidant activity [9,17,18]. Sericin has the potential to be used instead of FBS in the culture media, as reported previously for different cell types such as human adipose tissue-derived stem cells [19], myeloma cell lines, ovarian cells, fibroblasts, keratinocytes and insect cell lines [20]. Furthermore, Hosoe et al. [21] reported that the addition of sericin instead of FBS to maturation medium enlarged the perivitelline space, increased hyaluronan (hyaluronic acid) production and decreased polyspermic fertilization in bovine oocytes. To the best of our knowledge, few attempts have been made to culture ovine oocytes and embryos in serum-free media containing silk protein sericin. Therefore, in the present study, we performed a series of experiments to test various sericin concentrations in our IVM and IVC systems, as a possible means of removing the detrimental effects of serum.

2. Material and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

2.2. Collection of sheep oocytes and in vitro maturation (IVM)

Oocyte collection and maturation were performed as previously described [11]. Briefly, ovine ovaries were obtained from an abattoir and transported to the laboratory within 1–2 h in a 0.9% saline solution, at 35–37 °C temperature. Follicles with diameters between 2 and 6 mm were aspirated with a 21 gauge needle attached to a 5 mL syringe. Only cumulus-oocyte complexes with compact cells and homogeneous cytoplasm were selected for IVM. Groups of 6–7 cumulus-oocyte complexes were cultured in 50 μL droplets of IVM medium incubated in a humidified incubator maintained at 38.5 °C and 5% CO_2 in air. The basic oocytes maturation medium consisting of [TCM-199 (Earle's Salts with L-glutamine and sodium bicarbonate; Gibco, UK) with 0.5 μg/mL FSH, 5 μg/mL LH, 1 μg/mL estradiol 17-β, 50 μg/mL of gentamycin sulfate], under mineral oil. Oocytes were kept for 22–24 h in a humidified incubator maintained at 38.5 °C and 5% CO_2 in air.

2.3. Oocyte maturation assessment: cumulus expansion and nuclear stage

Culture was terminated after 24 h, and degree of cumulus expansion was assessed. Full cumulus expansion was characterized by extremely sticky nature of the cumulus mass to at least three diameters (>300 μm) away from the zona pellucida. The expansion of the cumulus to two diameters (200 μm) was classified as moderate expansion while slight expansion was characterized by cumulus cells being tightly adherent to the zona. Oocyte nuclear stage in meiosis was determined after aceto-orcein staining. Briefly, the oocytes were denuded by gentle pipetting and then fixed for at least 24 h in ethanol fixative solution (1:3). Afterward, they were placed on a clean glass slide and overlaid with a square cover slip that was held up by four droplets of a vaseline-paraffin mixture (40:1). Thereafter, the oocytes were stained for 1 min with 1% aceto-orcein before washing with a mixture of distilled water, glycerol, and acetic acid (3:1:1). Finally, the stages of nuclear maturation including GV, GVBD, MI, A-T and MII, were recorded under a phase contrast microscope (Olympus B201; Olympus) and only MII oocytes were considered as mature oocytes [22].

2.4. Sperm preparation and fertilization

In both experiment, fresh semen of a ram of known fertility was utilized for IVF. The motility of the sperm cells was evaluated under an inverted microscope and the motile sperm were separated using the swim up technique as described [22–24]. After IVM, COCs were partially denuded of granulosa cells by gentle pipetting, and then washed three times in the fertilization medium (TALP). Groups of 5–7 oocytes were transferred into the 48 μL fertilization droplets. Insemination was carried out by adding 1–2 × 10^6 spermatozoa/mL, 2 μg/mL heparin, and PHE (penicillamine, 20 μmol/L; hypotaurin, 10 μmol/L; epinephrine, 1 μmol/L). The mixture of gametes was incubated with spermatozoa for 6–7 h at 38.5 °C and 5% CO_2 in humidified air atmosphere.

2.5. In vitro culture (IVC)

The presumptive zygotes were taken from the fertilization drops after 6–7 h of co-incubation. After washing, the presumptive zygotes were then placed in 60 μL of KSOM-aa in a 60 mm Petri dish, covered with mineral oil and incubated until day 8 after fertilization at 38.5 °C in a humidified atmosphere of 5% CO_2. To prevent toxic accumulation of ammonium as a result of amino acid degradation, KSOM-aa medium was replaced every 48 h. Cleavage was assessed after 48 h of culture, and the numbers of embryos developing to the morula and blastocyst stages were assessed on days4 and 6, respectively (day 0 = day of IVF). In the present study, a two step-culture system was used. The first KSOM-aa1 medium contained 0.8% BSA fraction V was used for the first 48 h. Then, the medium was replaced by the second step (KSOM-aa2) containing 0.8% FOS for the remaining 6 days of culture.

2.6. Experimental design

2.6.1. Experiment 1: effects of various sericin concentrations, PVA and FOS on in vitro maturation

In the first experiment, COCs were matured in the presence of the basic oocytes maturation medium plus 10% FOS, 0.1% PVA and different concentrations of sericin (0.1, 0.5, 1 and 2.5%), for 24 h. Followed by fertilization and culture, as described above. Data were collected on cleavage rate and development to the blastocyst stage on day 6–8 for each treatment.

2.6.2. Experiment 2: effects of various sericin concentrations, PVA and FOS on in vitro culture

In the second experiment, presumptive zygotes were cultured in the presence of the first 0.8% BSA (control), 0.1% PVA and different concentrations of sericin (0.1, 0.5, 1 and 2.5%), for the first 48 h, then, 10% FOS (control), 0.1% PVA and different concentrations of sericin (0.1, 0.5, 1 and 2.5%), for 6 days of culture. The percentage of cleavage rate and cleaved zygotes developing to the blastocyst stage on day 6–8 was investigated.