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Effect of sericin supplementation in maturation medium on cumulus cell expansion, oocyte nuclear maturation, and subsequent embryo development in Sanjabi ewes during the breeding season

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ABSTRACT

The purpose of this study was to evaluate the effect of sericin with different concentrations (0% [control], 0.1%, 0.5%, 1.0%, and 2.5%) added to the IVM medium on cumulus cell expansion, oocyte nuclear maturation, and subsequent embryo development in Sanjabi ewes during the breeding season. The resumption of meiosis was assessed by the frequency of germinal vesicle breakdown and the first polar body extrusion. After IVF with fresh ram semen, presumptive zygotes were cultured 8 days in potassium simplex optimization medium supplemented by amino acids, and the percentages developing to the two-cell and blastocyst stages were measured as the indicators of early embryonic developmental competence. More cumulus–oocyte complexes matured with 0.5% sericin underwent germinal vesicle breakdown and reached metaphase II stage compared with the control cumulus–oocyte complexes matured without sericin ($P \leq 0.05$). The present findings indicated that supplementation with 0.5% sericin during the maturation culture may improve the nuclear maturation and the cumulus cell expansion. Furthermore, the percentage of blastocysts obtained from 0.5% and 0.1% sericin (37.8 ± 1.76% and 34.8 ± 1.09%, respectively) was higher ($P \leq 0.05$) than that of the control medium (29.60 ± 1.67%). However, addition of 1% and 2.5% of sericin to the IVM medium oocytes had a negative effect on nuclear maturation and cumulus cell expansion. Furthermore, the percentage of cleavage and blastocyst rate was significantly lower in the 1% and 2.5% sericin groups than in the control group. These findings showed that supplementation of IVM medium with 0.5% sericin may improve the meiotic competence of oocytes and early embryonic development in Sanjabi ewes during the breeding season.

1. Introduction

In vitro embryo production (IVEP) technologies including IVM, IVF, and *in vitro* embryo culture are considered the key technologies in animal reproduction and biomedical fields [1]. However, the success of IVEP is closely related to the IVM success rate, which is then one of the limiting steps. Consistently, successful and reliable oocyte maturation (both cytoplasmic and nuclear maturation) would dramatically improve the efficiency of preimplantation embryonic development and fetal development [2]. As with other technologies, IVEP technologies have their share of problems and failures [3]. Oocytes collected from growing follicles for the IVEP are blocked at the prophase stage of meiosis I. Meiotic resumption of oocytes outside the follicular inhibitory environment with progression to the metaphase II (MII) stage, in an essentially standard cell culture *in vitro* condition [4], is the basis of IVM. Finding one or more materials that can maximize the

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maturation rate and improve the quality of *in vitro*–matured oocytes has been the focus of research in this area around the world [5]. Reports in the literature have described the effect of different types and concentrations of materials including gonadotrophic hormones, growth hormone [6], growth factors [7], glucose, lactate, and pyruvate [8] to the sheep oocyte culture medium.

Sericin is one of the major components of silks of mulberry as well as nonmulberry silkworms and, although presently underutilized, it has potential utility in pharmacologic, cosmetics, and biotechnological applications [9]. Sericin is a water-soluble component of silk that covers fibroin fibers and fixes them to each other in cocoons. Minoura et al. [10] found that sericin enhanced the attachment and growth of mouse fibroblasts when used as a substratum. Then, Terada et al. [11] found growth promotion in several human cell lines and mouse hybridoma when they added sericin to the culture media. In another study, Tsubouchi et al. [12] found that sericin M, the main component among three molecular species of cocoon sericin, could enhance the attachment of primary cultured human skin fibroblasts. The attachment and subsequent proliferation of skin fibroblasts were considered to play important roles in the healing process of skin lesions. Sasaki et al. [13] indicated that sericin, particularly sericin S, could improve serum-free mammalian cell culture. Dash et al. [14] reported that the silk protein sericin from the nonmulberry tropical tasar silkworm, *Antheraea mylitta*, can serve as a valuable antioxidant.

Regarding use of sericin in reproductive biotechnologies, Isobe et al. [15] reported that sericin can prevent oxidative stress during bovine embryo culture and thus result in the improvement of the embryo quality and increased embryonic development. Furthermore, Do et al. [16] reported that supplementation of 1.0% sericin during maturation culture had a slight effect on the nuclear maturation and fertilization of porcine oocytes and the quality of the embryos but did not affect blastocyst formation. Also, Hosoe et al. [17] reported that the addition of sericin, instead of fetal bovine serum (FBS), to maturation medium enlarged the perivitelline space, increased hyaluronic acid (HA) production, and decreased polyspermic fertilization in bovine oocytes. Yasmin et al. [18] indicated that 0.1% sericin supplementation can improve the rates of maturation and total fertilization of sheep oocytes cultured in a maturation medium supplemented without BSA. These findings suggested that sericin, as an alternative protein supplement for IVM, plays a beneficial role in the ooplasmic maturation of bovine oocytes [17].

However, there is apparently no available information concerning the effects of silk protein sericin on the development of sheep embryos cultured *in vitro*. This study was thus conducted to examine whether the supplementation of the IVM medium with sericin would improve the nuclear maturation, fertilization, and development of the Sanjabi ewes oocytes.

2. Materials and methods

In the present study, all the chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), and plastics were purchased from Falcon (Paignton, UK) unless otherwise indicated. The ovine serum was heat inactivated at 56 °C for 30 minutes in a water bath, dispensed into 1-mL aliquots, and stored at −20 °C until being used. Except the media with FSH or LH, all other solutions were filter sterilized (0.22-μm sterilizing filter; Sartorius, Germany) before use.

2.1. Oocyte collection and in vitro maturation

Ovaries of pubertal Sanjabi ewes were collected during the breeding season from a local abattoir and transported to the laboratory in sterile 0.9% NaCl containing 100 IU/mL of penicillin and 100 mg/mL of streptomycin at 35 °C to 37 °C, arriving at the laboratory within 2 hours. The ovaries were washed for three times in the warm saline containing 50 IU/mL of penicillin and 50 μg/mL of streptomycin, and the extraneous tissues were removed. N-2-hydroxyethylpiperazine-N-2′-ethanesulfonic acid (HEPES)–buffered tissue culture medium 199 (TCM-199) supplemented with 4 mg/mL of BSA, 25 IU/mL of heparin, and 50 μg/mL of gentamicin sulfate was used for handling oocytes before and after the culture. Oocytes were liberated from follicles with the diameter of 2 to 6 mm [5]. The cumulus–oocyte complexes (COCs) were isolated under a stereomicroscope (Nikon Corporation, Japan) and graded as good, fair, or poor [19]. Only good or fair oocytes were considered acceptable and used in IVM experiments. The selected oocytes were then subjected to maturation in the following media: TCM-199 (Earle's salts with L-glutamine and sodium bicarbonate; Gibco, UK) with 0.5 μg/mL of FSH, 5 μg/mL of LH, 1 μg/mL of estradiol 17-ß, 50 μg/mL of gentamicin sulfate, and 10% fetal ovine serum, supplemented with 0% (control), 0.1%, 0.5%, 1.0%, and 2.5% (wt/vol) sericin. The COCs were incubated in a tissue culture dish (NUNC; VWR International, Milan, Italy) for 24 hours at 38.5 °C at the humidified atmosphere of 5% CO_2 in air.

2.2. Oocyte maturation assessment: Cumulus expansion and nuclear stage

Cumulus expansion was determined at 24 hours by the subjected descriptions as not expanded, partially expanded, or fully expanded under a stereomicroscope (Olympus SZH, DF Plan 1X; Olympus). Oocyte nuclear stage in meiosis was determined after aceto-orcein staining [20]. Briefly, the oocytes were denuded by gentle pipetting and then fixed for at least 24 hours in ethanol fixative solution (1:3). Afterward, they were placed on a clean glass slide and overlaid with a square coverslip that was held up by four droplets of a vaseline–paraffin mixture (40:1). Thereafter, the oocytes were stained for 2 minutes with 1% aceto-orcein before washing with a mixture of distilled water, glycerol, and acetic acid (3:1:1). Finally, the nuclear maturation was recorded under a phase contrast microscope (Olympus B201; Olympus) [21].

2.3. Sperm preparation and fertilization

In this experiment, fresh semen of a ram of known fertility for IVF was used. The motile sperm was separated using the swim-up technique as described [21–23]. After maturation, the COCs were partially denuded of granulosa