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Short communication

In vitro culture medium (IVC) supplementation with sericin improves developmental competence of ovine zygotes

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ABSTRACT

This study was carried out to investigate the effects of supplementation of potassium simplex optimized medium (KSOM-aa) with various sericin concentrations (0, 0.1, 0.5, 1 and 2.5%) on ovine zygotes. The results indicate that the supplementation of oocyte *in vitro* culture medium with optimal concentration of sericin (0.1 and 0.5%) may have beneficial effects on developmental competence of *in vitro*-derived ovine embryos.

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1. Introduction

In vitro production of embryos (IVEP) provides a good opportunity to obtain an inexpensive and abundant number of embryos for conducting basic research and for application of emerging biotechnologies, *e.g.*, cloning and transgenesis [1]. IVEP technologies including *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* embryo culture (IVC) are considered key steps in the field of animal reproduction and biomedical research [2]. In sheep, nearly 85% of matured oocytes are fertilized after IVF. However, only approximately 30% of zygotes develop to the blastocyst stage after 8 days of *in vitro* culture [3]. Exposure to suboptimal environments at the preimplantation stages of development may disrupt the normal developmental program. Therefore, the media for IVC of ovine zygotes must be carefully optimized to improve the efficiency of *in vitro* embryo production.

Sericin is a water-soluble member of the family of serine (Ser)-rich silk proteins of mulberry as well as non-mulberry silkworms that glues fibroin fibers together to form a robust cocoon [4]. It accounts for 20–30% of weight of the *Bombyx mori* cocoon fiber and is usually removed by the degumming process during silk processing. Currently, in some laboratories

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sericin is used as a replacement for fetal bovine serum (FBS) in cell culture media [5]. It was reported that sericin stimulated the proliferation of insect and mammalian cells and improved the viability of many cell lines following cryopreservation [5]. Also, Hosoe et al. [6] reported that the addition of sericin instead of FBS to maturation medium enlarged the perivitelline space, increased hyaluronic acid (HA) production and decreased polyspermic fertilization in bovine oocytes. These findings suggest that sericin may be used not only as a FBS replacement, but also as a supplementation of culture medium containing FBS. However, information concerning the effect of various concentrations of sericin on *in vitro* embryo development in ovine is scarce. Therefore, the objective of the current study was to determine whether potassium simplex optimized medium (KSOM-aa) supplemented with different concentrations of sericin improves the cleavage rate and subsequent embryonic development *in vitro*. Further studies are necessary to evaluate the exact role and mechanism of sericin action on ovine oocyte maturation and embryonic development.

2. Materials and methods

All chemicals and cell culture media were purchased from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise stated. All plastic ware were obtained from Falcon Becton Dickinson (Franklin Lakes, NJ, USA). Sanjabi ewe ovaries were obtained from a local abattoir and were transported in a thermo flask containing normal saline (30–35 °C), fortified with penicillin (400 IU/mL) and streptomycin (50 μg/mL). All ovaries were washed with saline. The follicles visible on the surface (2–6 mm diameter) were aspirated using a sterile 21 gauge needle into an oocyte collection medium, consisting of TCM-199 supplemented with 25 mM HEPES + 0.3% bovine serum albumin (BSA). All oocytes were washed 5–6 times with washing medium consisting of TCM-199 supplemented with 50 μg/mL gentamycin and 10% (v/v) fetal ovine serum (FOS). The cumulus oocyte complexes (COC's) with ≥3 layers of compact cumulus cells and a homogeneous ooplasm were used in the experiment.

After oocyte classification, the COCs were washed three times in a maturation medium (TCM-199; Earle's Salts with L-glutamine and sodium bicarbonate; Gibco, Waltham, MA, USA) supplemented with 0.5 μg/mL FSH, 5 μg/mL LH, 1 μg/mL estradiol 17-ß, 50 μg/mL of gentamycin sulfate and 10% (v/v) FOS). The COCs were incubated in a tissue culture dish for 24 h at 38.5 °C in a humidified atmosphere of 5% CO_2 in air.

Fresh semen of a ram with known fertility was used for IVF. The motility of sperm cells was evaluated under an inverted microscope and the motile spermatozoa were separated using the swim up technique [7]. After IVM, the COCs were partially denuded of granulosa cells by gentle pipetting, and then washed three times in 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; hypotaurine, 10 μmol/L; epinephrine, 1 μmol/L). Oocytes were co-incubated with spermatozoa for 6–7 h at 38.5 °C and 5% CO_2 in humidified air atmosphere.

After 6–7 h of co-incubation, presumptive zygotes were denuded from remaining cumulus cells by gentle pipetting. After washing, the presumptive zygotes were cultured in groups of 10–15 in 60 μL droplet of KSOM-aa medium and cultured until day 8 after fertilization at 38.5 °C in a humidified atmosphere of 5% CO_2. The culture medium was refreshed every 48 h. Cleavage was assessed after 48 h of culture, and the numbers of embryos developing to the morula and blastocyst stages was assessed on days 4 and 6, respectively (day 0 = day of IVF). In the present study, a two step-culture system was used. The first KSOM-aa medium (KSOM-aa1) contained 0.8% crystallized BSA was used for the first 48 h. Then, the medium was replaced by the second KSOM-aa medium (KSOM-aa2) containing 0.8% FOS and used for the remaining 6 days of culture.

After maturation and fertilization, presumptive zygotes were cultured in KSOM-aa medium supplemented with different concentrations of sericin. The *ex vivo*-fertilized zygotes were assigned to a control, *i.e.*, sericin-unexposed, group (treatment1; T1) and the following groups of *in vitro* cultured embryos exposed to 0.1% (treatment 2; T2), 0.5% (treatment 3; T3), 1% (treatment 4; T4) or 2.5% (treatment 5; T5) sericin. Each experiment was repeated five times. The statistical analysis was performed using SAS program package (SAS for Windows, version 9.1). The cleavage, morula and blastocyst rates among the treatment groups were checked for normal distribution using Proc Univariate. If required, we used data conversion (log of data) then analysis of variance (ANOVA) was performed on new data (normally distributed) using the Proc GLM. The Duncan's multiple range test was used to test the differences between the treatments. Values were expressed as means ± SD and $p < 0.05$ was considered the significant level.

3. Results and discussion

This is the first study reporting that the development of ovine embryos was effectively promoted by sericin. Silk sericin exhibited protective effects against oxidative stress in cell lines, such as fibroblasts [8], bovine embryos [9] and porcine oocytes [10]. Similar preparations accelerated the proliferation of mammalian cells [11], improved their performance in the serum-free cultures [5], and enhanced attachment of the cultured human skin fibroblasts [12].

The embryonic development of 347 ovine presumptive zygotes exposed to sericin ($n = 279$) and 68 zygotes served as controls (cultured only in the medium without sericin) were examined in the study (Table 1). The cleavage rate was significantly ($p < 0.05$) higher in the 0.1 and 0.5% sericin groups than in the control group. After 8 days of culture, the blastocyst rate in these groups was also significantly higher ($p < 0.05$) than in controls. In contrast, when embryos were cultured in the presence of high concentration (2.5%) of sericin, the cleavage, morula and blastocyst rates were lower ($p < 0.05$) compared with the control group (Table 1).

The adenosine triphosphate (ATP) synthesis rate is increased at the time of blastocyst formation to support rapid protein synthesis and elevated activity of membrane ion-transport [13]. An increased uptake of oxygen and energy

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