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# Effect of 2-mercaptoethanol and cysteine supplementation during in vitro maturation on the developmental competence of oocytes from hormone-stimulated lambs

J. Bai, J. Hou, H. Guan, F. Yan, X. Cui, L. Liu, S. Wang, X. An *

*State Key Laboratory for Agrobiotechnology and Department of Animal Physiology, College of Biological Science, China Agricultural University, Beijing 100094, China*

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**Abstract**

The objective was to determine the effect of 2-mercaptoethanol and cysteine on in vitro developmental competence of oocytes from lambs (4–8-week old) stimulated with eCG and pFSH. Oocytes were matured in medium (TCM199) with no supplement (Control group) or with 100 μM 2-mercaptoethanol and 600 μM cysteine (GSH group). Oocytes from adult sheep were also included (Adult group). The addition of 2-mercaptoethanol and cysteine did not improve nuclear maturation or microtubule configuration 12, 15, 18, or 24 h after placement in maturation medium. Sperm head decondensation and male pronucleus formation were evaluated at 6, 12, and 18 h after commencement of IVF; sperm decondensation appeared earlier in the GSH group (6 h after the start of IVF). There were differences ($P < 0.05$) between the Control group and the GSH and Adult groups for: fertilization rate at both 12 h (55.4, 77.0, and 80.6%, respectively) and 18 h (67.9, 86.9, and 88.7%); parthenogenesis rate at both 12 h (25.0, 10.8, and 5.6%) and 18 h (28.3, 9.8, and 4.5%); and polyspermy rate at 18 h (26.4, 4.9, and 5.7%). Blastocyst rate at 7 d was higher in the GSH group than the Control group (23.9% vs. 14.9%, $P < 0.05$), but both were lower ($P < 0.05$) than the Adult group (38.3%). The addition of 2-mercaptoethanol and cysteine improved sperm decondensation and rates of fertilization and the blastocyst development to 7 d, with no effect on blastocyst rate at 9 d.
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*Keywords:* Prepubertal; Oocyte; Ooplasmic maturation; 2-Mercaptoethanol; Cysteine

## 1. Introduction

It is possible to harvest large numbers of oocytes and to produce live offspring from hormonally stimulated prepubertal calves [1,2] and lambs [3,4]. Although most oocytes from prepubertal animals resume meiosis in response to exogenous hormones and complete nuclear maturation in vitro, developmental competence is still significantly lower than that of oocytes collected from adults (bovine [5,6], ovine [7,8], pig [9], and goat [10,11]). It was previously reported that prepubertal oocytes were smaller in diameter (bovine [12] and ovine [7]), with delayed maturation, abnormal chromatin and microtubule configurations, low rates of protein synthesis, high rates of parthenogenesis and polyspermy, impaired sperm chromosome decondensation, asynchronous pronucleus formation, and low rates of blastocyst formation during in vitro maturation, fertilization and embryo culture (ovine [7,8], bovine

* Corresponding author at: College of Biological Science, China Agricultural University, No. 2 Yuan-Ming-Yuan West Road, Haidian District, Beijing 100094, China. Tel.: +86 10 62733463; fax: +86 10 62733463.
*E-mail address:* xra@cau.edu.cn (X. An).

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[12–15], and goat [10,16,17]). It was speculated that all of these problems could be due to deficiencies in cytoplasmic maturation [18].

The reducing agent glutathione (GSH) has several important roles during oocyte development, including protection from oxidative damage, maintenance of the meiotic spindle and improving both chromatin decondensation and male pronucleus formation [19]. The GSH content increased during oocyte maturation; furthermore, oocytes matured in vivo contained significantly more GSH than oocytes matured in vitro [20–23]. That GSH concentration was lower in oocytes from prepubertal animals than in oocytes from adult animals [15,24] was particularly noteworthy. Several studies have examined the effects of adding thiol-containing precursors of GSH including cysteamine, 2-mercaptoethanol, cysteine, and cystine to media to assist in the production of embryos in vitro (bovine [15,20], ovine [21], pig [22], goat [24], and hamster [23]). In general, these compounds increased the GSH concentration and improved sperm decondensation and pronucleus formation.

Supplementation with 2-mercaptoethanol and cysteine during the in vitro maturation of oocytes from adult sheep increased intracellular GSH concentrations [21]. Extracellular cysteine can be transported into the oocyte and 2-mercaptoethanol promoted the uptake of cysteine and synthesis of GSH. However, the effects of these supplements during maturation on the development of the lamb oocyte are unknown. Consequently, in this study, we determined the effects of adding 2-mercaptoethanol (100 μM) and cysteine (600 μM) on chromatin and microtubule configuration during in vitro maturation, sperm decondensation and male pronucleus formation after fertilization and on the blastocyst rate after in vitro culture.

## 2. Materials and methods

### 2.1. Chemicals and materials

Dishes for oocyte culture were purchased from Nunc Company (Nunc Inc., Naperville, IL, USA), and all chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated.

### 2.2. Oocyte collection

Ovaries from 1 to 2-year-old Mongolian sheep were collected at a local abbatoir and Mongolian lambs were hormonally stimulated at 4–8-week of age. The hormone treatment consisted of 4× 40 mg FSH (Folltropin; Bioniche Inc., Belleville, ON, Canada) per lamb, given IM at approximately 12 h intervals and 400 IU of eCG (Huafu Inc., Tianjin, China) per lamb given concurrently with the first FSH treatment [4]. Oocytes were collected approximately 48 h after the first FSH treatment. Ovaries were obtained by ovariectomy under anaesthesia induced by 100 mg of ketamine (The Academy of Military Medical Sciences, Changchun, China) and transported to laboratory (within 1 h) in 0.9% sodium chloride solution containing streptomycin and penicillin (temperature, 37 °C). After three washes in 0.9% sodium chloride solution containing penicillin/streptomycin, antral follicles (approximately 4–6 mm in diameter) were aspirated using a 10 mL syringe and an 18 ga needle containing Hepes-TCM199, supplemented with 2% heat-inactivated sheep serum, 50 μg/mL heparin, 100 μg/mL streptomycin, and 100 IU/mL penicillin. Oocytes with more than one complete layer of unexpanded cumulus cells were selected for in vitro maturation.

### 2.3. In vitro maturation, fertilization and culture

Oocyte–cumulus complexes (OCC) were rinsed three times in Hepes-buffered TCM199 containing 2% sheep serum and then twice in maturation medium. The maturation medium for the Control group was sodium bicarbonate-buffered TCM199 containing 20% heat-inactived estrous sheep serum, 5 μg/mL FSH (Bioniche Canada), 5 μg/mL LH (Bioniche Canada), 1 μg/mL estradiol 17β, 100 μg/mL streptomycin, and 100 IU/mL penicillin. For the GSH group, the maturation medium was supplemented with 100 μM 2-mercaptoethanol and 600 μM cysteine, according to published information [20,24]. Lamb oocytes were allocated, within individual animals, to the Control and GSH groups. The OCC were matured in four-well dishes with each well containing 600 μL maturation medium and covered with 300 μL of mineral oil. There were 30–40 OCC per well and maturation occurred for 24 h at 38.5 °C in a humidified atmosphere of 5% $CO_2$ in air.

After maturation, the OCC were stripped of excess cumulus cells by gentle pipetting in 0.1% hyaluronidase, and then washed three times in fertilization medium. The fertilization medium was synthetic oviduct fluid (SOF) containing 2% sheep serum. Approximately 30–40 oocytes were placed into each well (that contained 450 μL fertilization medium, covered with 300 μL mineral oil). Motile sperm were obtained using the ‘‘swim up’’ procedure; 200 μL of frozen-thawed semen were layered below 600 μL IVF

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