



Effect of Glutathione Supplementation on *in vitro* Maturation of Oocytes in Goats

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ABSTRACT

Oxidative stress is a significant impediment to *in vitro* embryo production. Glutathione (GSH) is among the strongest antioxidants. This study aimed to assess the effect of adding GSH to the *in vitro* maturation (IVM) medium on the maturation rate and oxidative status of goat oocytes during IVM. The goat oocytes (n=372) were divided into a control (T1; without GSH) group, and experimented groups (0.5, 1, and 2 mM/mL of GSH, T2, T3, and T4 respectively). The maturation rate, assessed by expansion of cumulus cells, and 1st polar body extrusion, was evaluated. Additionally, the oxidative status of oocytes post IVM was assessed through total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels. The current study's findings indicated that the cumulus cells expansion and first polar body extrusion rates were (74.26% and 24.75%), (80.90% and 34.52%), (91.4% and 43.01%) and (84.27% and 30.34%) for T1, T2, T3, and T4, respectively. Moreover, the results of the antioxidant enzymes activities showed that MDA level was reduced ($P<0.05$) in T3 compared to other groups. Also, the TAC was higher ($P<0.05$) in T3 in comparison with other groups. Moreover, the concentration of GPx, was higher in T3, and T4 compared to other groups. However, no difference was found in the SOD level between the experimental groups. In conclusion, these results indicate that supplementing the oocyte maturation medium with glutathione (GSH), especially at concentration of 1 mM/mL can improve IVM rate, and the antioxidant capacity of goat oocytes.

Keywords: Glutathione, antioxidant, oocyte, *in vitro* maturation, goat.

1. Introduction

Numerous studies have been done on the detrimental effects of oxidative stress on oocyte's *in vitro* maturation (IVM) and embryo development (Guerin *et al.*, 2001 & de Matos *et al.*, 2003). Oxidative processes resulting from the overproduction of reactive oxygen species (ROS) can also harm the sperm, affecting their structure and function (Gadea *et al.*, 2004). Numerous investigations have been carried out to assess how antioxidants affect the maturation of oocytes and the early stages of embryonic development (Urdaneta *et al.*, 2003; Rodriguez-Gonzalez *et al.*, 2003; Feugang *et al.*, 2004; Khattab *et al.*, 2020 and Ashour *et al.*, 2021). In mammalian cells, glutathione (GSH) is a non-protein antioxidant (-SH) (G-Glu-Cys-Gly) that is crucial for shielding cells from oxidative damage. In mice, hamsters, pigs, and cattle, GSH synthesis has been documented during oocyte maturation. In the ovary, Glutathione levels rise during the growth and maturation of oocytes, as they approaching ovulation. Following fertilization, GSH is engaged in sperm condensation together with oocyte activation, in addition to the transform of the sperm genetic content to the male nuclei (de Matos *et al.*, 1997).

According to Lafleur *et al.* (1994), glutathione has been reported as having multiple activities, including an effect on amino acid transport, DNA and protein synthesis, and bisulfite reduction. Moreover, semen naturally contains glutathione, which shields sperm from oxidative damage caused by the large amount of ROS created during freezing and thawing (Gadea *et al.*, 2007). Prior research found that adding 2 mM glutathione to the cryoprotectant increased the cryo-preservability and improved the *in vitro* fertilization potential of sperm by protecting DNA from degradation and reducing

oxidative stress in bovine (Badr *et al.*, 2012). It was also reported that higher intracellular concentrations of GSH enhanced the oocytes' IVM rate in buffalo (Gasparrini *et al.*, 2006).

In goats, Mayor *et al.* (2001) found that adding 1 mM/mL of GSH might be a helpful method to increase GSH levels in goat oocytes during IVM. Furthermore, they found that adding the same concentration during *in vitro* fertilization significantly increased the fertilization rate of goat oocytes (Urdaneta *et al.*, 2004). The current study sought to determine how the *in vitro* maturation rate of goat oocytes was affected by the addition of varying quantities of glutathione to the *in vitro* maturation medium.

2. Materials and Methods

2.1. Work location

The study was conducted at the Embryo Manipulation Unit (EMU), Desert Research Center (DRC), Cairo, Egypt.

2.2. Oocytes *in vitro* maturation

All media were obtained from Sigma-Aldrich (St. Louis, MO, USA) or otherwise noted. The goat IVM procedure was conducted according to Abdelkhalek *et al.* (2024). The goat ovaries were collected from local slaughter houses near Cairo, transported to the lab in a thermos container filled with saline solution (35 °C) fortified with gentamycin 50 µg/mL within two hours after slaughter.

Oocytes (n= 372) were recovered from goat ovaries using a slicing technique. The oocytes were washed three times using wash medium (WM) consisted of TCM-199 with HEPES, 10% FBS (v/v) and 50 µg/mL gentamycin. This was then followed by a final wash with IVM media which consisted of TCM-199, 10% FBS, 20 IU/mL equine Chorionic Gonadotropin (eCG, Gonaser®, 500 IU), 20 IU/mL human Chorionic Gonadotropin (hCG, Choriomon®, 5000 IU), 0.25 mg/mL Na⁺ pyruvate, 50 µg/mL gentamycin, 10 ng/mL epidermal growth factor (EGF), and 1 µg/mL estradiol (E2). Groups of 20-25 oocytes per group were cultured in 100 µL drop of the prewarmed 38.5 °C IVM medium each under mineral oil in a CO₂ incubator 5% CO₂, with 95% humidity for 24 hours.

2.3. Experimental design

The experimental groups differed in glutathione concentrations in maturation media; T1: control group without GSH. T2: 0.5 mM/mL GSH. T3: 1 mM/mL GSH. T4: 2 mM/mL GSH.

2.4. Evaluation of oocyte maturation

After 24 h of IVM, the rate of maturation was assessed based on the cumulus cells expansion, and the first polar body extrusion (Fig. 1). In order to examine the first polar body, two minutes of moderate pipetting were applied to get rid of cumulus cells, and the oocytes were assessed under the inverted microscope (Leitz Fluovert FU Leica Microsystems, Wetzlar, Germany).

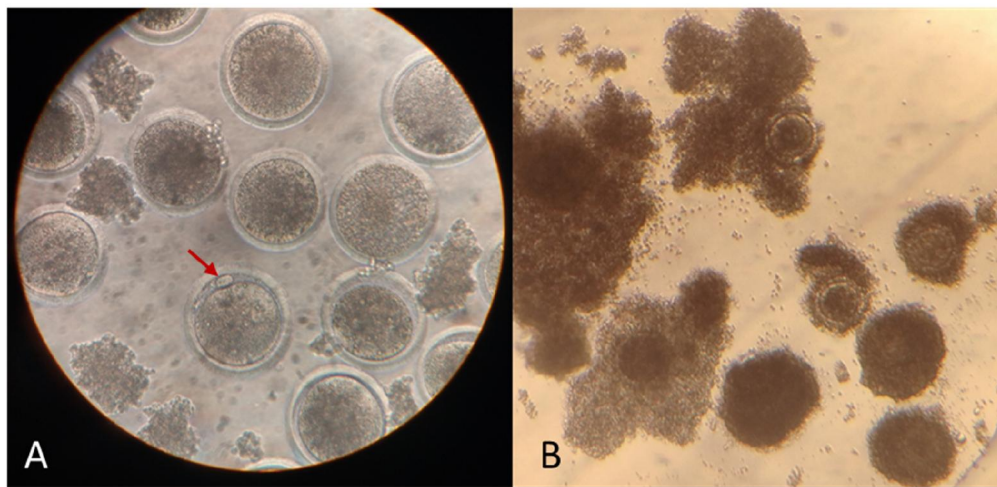


Fig.1: Mature goat oocytes. A. First polar body (red arrow). B. Expansion of cumulus cells.

2.5. Evaluation of oocyte Antioxidant Enzymes Activities

Post-maturation, mature oocytes contents were extracted using repeated cycles of freezing and thawing in liquid nitrogen (-196 °C). According to earlier reports (Koracevic *et al.* 2001; Satoh, 1978; Nishikimi *et al.* 1972; Aebi, 1984), the antioxidant enzyme activities of glutathione peroxidase (GPx Cat. # GP2524), malondialdehyde (MDA, Cat. # MD2529), superoxide dismutase (SOD, Cat. # SD2521), and total antioxidant capacity (TAC, Cat. # TA2513) were measured colorimetrically using commercial kits (Biodiagnostic Research, Egypt).

2.6. Statistical analysis

Data of maturation rates were statistically analyzed by a Chi square test, in addition, one-way ANOVA was performed for the same parameter (after the angular transformation of the data) to distinguish the significant differences between groups' means. While the data of antioxidant assay were analyzed by one-way ANOVA, using the SAS program, 2004 (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA).

3. Results

The results of goat oocytes *in vitro* maturation showed that 1 mM/mL concentration of GSH in T3 produced higher ($P < 0.05$) proportion of expanded cumulus cells and polar body extrusion (91.4% and 43.01%, respectively), compared to T1 (74.26% and 24.75%), T2 (80.90% and 34.52%), and T4 (84.27% and 30.34%).

Table 1. Effect of GSH supplementation on goat cumulus cells expansion during oocyte IVM

Treatment	No of oocytes	Expansion rate*	SE	Chi value (Prob)
T1	101	74.26 ^c (75/101)	1.47	10.2160 (0.0168)
T2	89	80.90 ^{bc} (72/89)		
T3	93	91.40 ^a (85/93)		
T4	89	84.27 ^b (75/89)		

Data presented as percentages. T1: control group without GSH. T2: 0.5 mM/mL GSH. T3: 1 mM/mL GSH. T4: 2 mM/mL GSH.

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

Table 2. Effect of GSH supplementation on polar body extrusion rate during goat oocyte IVM

Treatment	No of oocytes	Polar body rate*	SE	Chi value (Prob)
T1	101	24.75 ^b (25/101)	0.97	7.6996 (0.05)
T2	89	34.52 ^{ab} (29/89)		
T3	93	43.01 ^a (40/93)		
T4	89	30.34 ^{ab} (27/89)		

Data presented as percentages. T1: control group without GSH. T2: 0.5 mM/mL GSH. T3: 1 mM/mL GSH. T4: 2 mM/mL GSH.

*Mean values in the same column followed by the same letter are not significantly different at $p < 0.05$

The results of antioxidant enzymes activities in Fig 2 showed that malondialdehyde (MDA) concentration was reduced ($P < 0.05$) in T3 in comparison with other groups. In the same way, the TAC was higher ($P < 0.05$) in T3 in comparison with other groups.

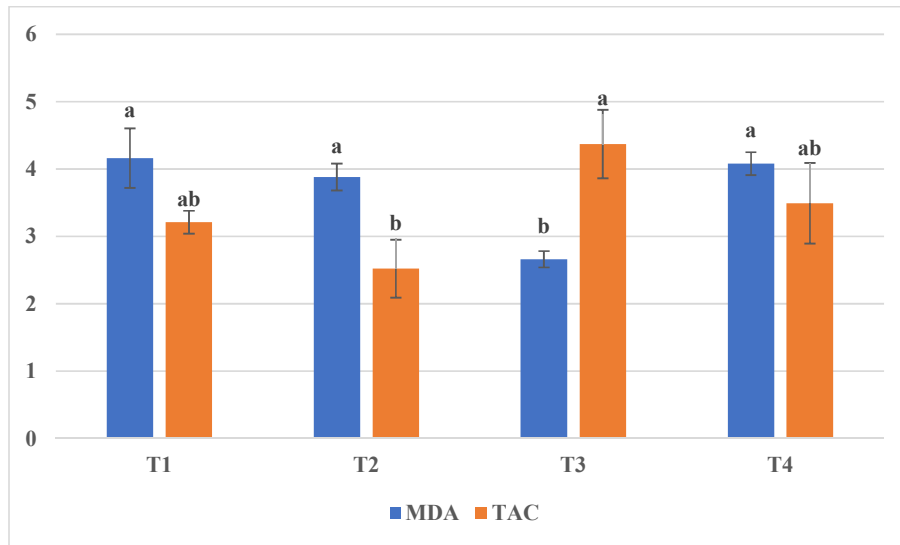


Fig. 2: Effect of GSH addition during IVM on malondialdehyde (MDA), and total antioxidant capacity (TAC) concentrations in goat oocytes. T1: control group without GSH. T2: 0.5 mM/mL GSH. T3:1 mM/mL GSH. T4: 2 mM/mL GSH.

Moreover, the results in Fig. 3 showed that the concentration of glutathione peroxidase (GPx), was higher in T3, and T4 compared to other groups. However, no difference was found in superoxide dismutase (SOD) concentration between the experimental groups.

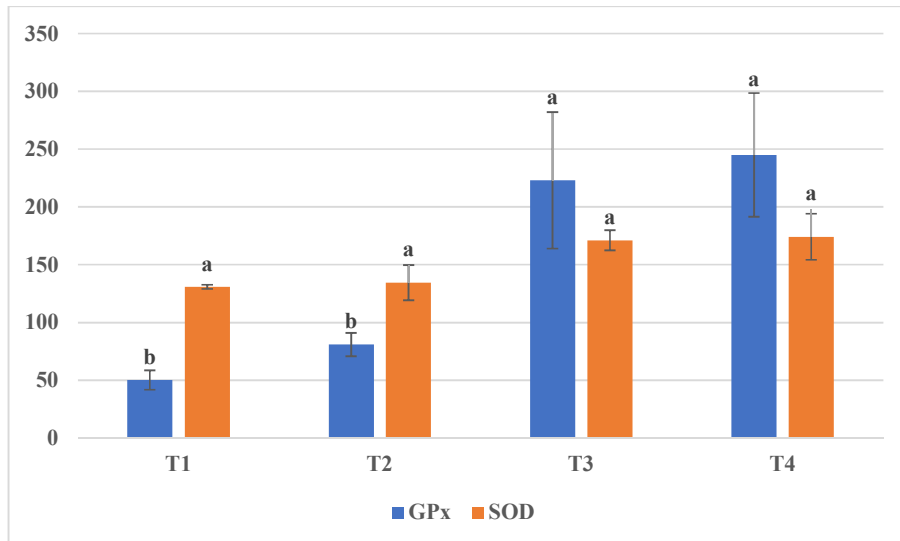


Fig. 3: Effect of GSH addition during IVM on glutathione peroxidase (GPx), and superoxide dismutase (SOD) concentrations in goat oocytes. T1: control group without GSH. T2: 0.5 mM/mL GSH. T3:1 mM/mL GSH. T4: 2 mM/mL GSH.

4. Discussion

The primary provider of mature oocytes used in research investigations was *in vitro* maturation. To increase the grade and yield of oocyte maturation and make IVM-produced oocytes competent in terms of fertilization and development, it was necessary to imitate the *in vivo* environment of oocyte maturation during *in vitro* culture (Zavareh *et al.*, 2016). Numerous studies have already demonstrated that oxidative damage during *in vitro* handling and cultivation, which is frequently observed in cell culture processes, is the primary factor affecting the maturation quality of oocytes. In order to combat oxidative stress, numerous studies have employed antioxidants as supplements, including vitamin C

(Ashour *et al.*, 2021), vitamin E (Campos Petean *et al.*, 2008), and β -mercaptoethanol (Rodriguez-Gonzalez *et al.*, 2003).

The glutathione (GSH) molecule contains a thiol group that has numerous physiological and metabolic functions. Additionally, GSH has a crucial function in maintaining the redox state in cells, which protects them from the detrimental effects of oxidative damage. Furthermore, various other actions of this substance have been stated, such as an effect on amino acid transport, DNA and protein synthesis, and disulfide reduction (Lafleur *et al.*, 1994). Moreover, it is engaged in various embryonic events and takes an important part in the advancement of embryos through species-specific "blocks" to development in mice (Gardiner and Reed, 1994). Therefore, the purpose of the current study was to assess the impact of adding various GSH dosages to goat oocyte maturation media *in vitro*.

The current research demonstrated that the incorporation of glutathione (GSH) into the IVM medium of goat oocytes enhanced the rate of maturation, attributed to cumulus expansion and the extrusion of the first polar body. In addition, the results of the antioxidant enzymes activities in mature oocytes treated with glutathione showed a dose-dependent effect. This was presented in the significant reduction of malondialdehyde (MDA) levels in the group treated with 1 mM/mL of GSH in comparison with other groups. Moreover, the total antioxidant capacity (TAC) was significantly higher in the same group (1 mM/mL of GSH) compared to the other groups. These findings are consistent with earlier investigations, which stated the significance of GSH in the IVM of oocyte and its subsequent development (Gasparrini *et al.*, 2003; de Matos and Furnus, 2000; Eppig, 1996; Funahashi, 1994). Moreover, GSH levels measured after IVM may serve as a trustworthy indication of cytoplasmic maturation, according to de Matos *et al.* (1997).

The findings of the present investigation may be elucidated by the significance of cytoplasmic glutathione (GSH), regarded as a key indicator of cytoplasmic maturation and playing a crucial function in safeguarding cells and gametes from oxidative stress (Luberda, 2005). In swine, GSH generated by cumulus cells appears to be transported across gap junctions, resulting in accumulation within the oocyte cytoplasm. (Mori *et al.*, 2000 and Nagai, 2001).

The extracellular matrix of cumulus, which is made up of glycosaminoglycans (Yokoo and Sato, 2004), may be crucial in preserving the cytoplasmic GSH level of IVM oocytes in addition to gap junctional connections. Hyaluronic acid and chondroitin sulfates, which are glycosaminoglycans, were found around somatic cells and are thought to play a role in protecting the cells from damage caused by oxidation in both *in vivo* and in *in vitro* environments (Campo *et al.*, 2004a, b).

5. Conclusion

Supplementing the oocytes' maturation medium with glutathione (GSH), especially a concentration of 1 mM/mL can enhance the *in vitro* maturation rate of oocytes, and the antioxidant capacity of goat oocytes.

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