In vitro fertilization of cryopreserved goat oocytes in different cryoprotectants

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Abstract. The aim of this research was to examine the rate of fertilization of in vitro matured goat oocytes using three different cryoprotectants. Iso-osmolarity concentrations of 1.5 M of ethylene glycol (EG), glycerol (GLY) and dimethyl sulfoxide (DMSO) were contrasted for their effectiveness as cryoprotectants. Ovaries were obtained from local abattoir and were brought to the laboratory in physiological saline 0.9% (w/v) at 25 to 30°C within 3 h. Follicular oocyte were collected by aspiration follicle with 2-6 mm diameter. This study exclusively selects oocytes with compacted ooplasm and cumulus cells. Following a 26h incubation, oocytes were cryopreserved and then fertilized. Data were pooled from 10 replicates. Statistical significance of differences among groups was analyzed using Chi-square analysis. Percentage of fertilized oocytes after freeze thawing in EG (48.38%) was higher (P<0.05) than in GLY (45.05%) or DMSO (44.50 %). The cleaved rate of cryopreserved oocytes in EG (25.28%) was higher (P<0.05) than of those frozen in GLY (19.28%) and DMSO (17.21%). This experiment suggests that EG was the most suitable cryoprotectant for cryopreservation of post-thaw in vitro matured goat oocyte.

1. Introduction
Oocyte freezing process requires a cryoprotectant to preserve cells viability after freezing. Cryoprotectant protects the cells, but also damages the cells due to its toxicity effect. The protection degree of cryoprotectant materials against crystallization process during freezing was determined by the type and concentration of cryoprotectant [1]. The objective of research was to evaluate the type of cryoprotectant on fertilization rate of matured goat oocytes.

2. Materials and Methods
2.1 Oocyte Preparation
Ovaries were obtained from goat at a local abattoir and were delivered to the laboratory in physiological saline 0.9% (w/v) at 25 to 30°C within 3 h. Cumulus oocyte complexes (COCs) were collected from follicular fluid aspirated through 18-G needle from 2-6 mm in diameter follicles. Subsequent to being washed twice with (TCM) 199 and once with maturation medium (TCM 199 with Earle’s salts; Gibco, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 1 ug/ml estradiol 17-B (Sigma, St Louis, USA) and antibiotics (100 µg/ml Streptomycin +100 IU /ml of Penicillin G, Meiji, Japan)[2] Oocytes with compacted ooplasm and cumulus cells were added into the maturation medium and covered with mineral oil, cultured for 26 h in 5% CO\textsubscript{2}, temperature 38.5°C.

2.2 Freezing, Thawing and In Vitro Fertilization (IVF)
The oocytes were cryopreserved in 1.5 M of ethylene glycol (EG), glycerol (GLY) and dimethyl sulfoxide (DMSO) for 10 minutes at room temperature, before it was normalized/neutralized for another 30 minutes at final solution. Next, oocytes were loaded in straw and then placed inside liquid nitrogen container at -196°C for two weeks. After that frozen straw would be thawed for analysis of morphology. The cryopreserved straws were placed in air for 5 s and then into a 37°C water bath for 30 s for thawing. Frozen thawed oocytes were fertilized with frozen semen according [3] After insemination, oocytes were fixed, stained with 1% aceto-orcein and observed under phase contrast microscope with magnification 400 x. Oocytes were considered to be fertilized when the female and male pronuclei with residual sperm tails were visible.

2.3 Statistical analysis
Data were pooled from 10 replications. Statistical significance of differences among groups were analyzed using Chi-square analysis (P<0.05).

3. Result and Discussion

Oocytes saturated in EG had a higher fertilization rate than oocytes frozen in GLY and DMSO. However, the fertilization rate of cryopreserved oocyte in GLY and DMSO were statistically lower (P<0.05) than fresh oocytes.

Table 1. In vitro fertilization of frozen thawed oocyte

| Cryoprotectant | No. (%) of oocyte | Polyspermy |
|----------------|------------------|------------|
|                | Examined         | Fertilized |                |
| Ethylene Glycol| 186              | 90(48.38%) | 17(16.19%)     |
| Glycerol       | 182              | 82(45.05%) | 19(20.43%)     |
| DMSO           | 182              | 81(44.50%) | 19(22.35%)     |
| Fresh oocyte   | 188              | 154(81.92%)| 21(13.64%)     |

Different superscripts within columns indicates significance (P< 0.05)

Proportion of morphologically normal frozen oocytes and cleaved oocytes in EG was significantly higher (P<0.05) than other cryoprotectants. However, it was also evident that cleaved oocytes of fresh oocytes were higher than cryopreserved oocytes. The differences in effects among three cryoprotectants was largely influenced by the difference in the weights of molecules, as EG had a relatively lower weight (62.07) in comparison to DMSO (78.13) and GLY (92.10), thus providing a more favourable impact on the oocytes’ permeability. Furthermore, EG was proven to be effective as a suitable cryoprotectant for cryopreservation due to its low degree of toxicity. It was evident from this study that the fertilization rate of frozen-thawed oocytes after insemination was lower than fresh oocytes. The reduced fertilization rate could be possibly caused by the damaged spindle, disorganization of plasma membrane, as well as structural changes in the zona pellucida during freezing [4,5].

Table 2. Cleaved rate of cryopreserved oocytes in different cryoprotectant

| Cryoprotectant | No. (%) of oocyte |
|----------------|------------------|
|                | Frozen | Normal | Cultured | Cleaved |
| Ethylene Glycol| 286    | 178(62.23%) | 178     | 45(25.28%) |
| Glycerol       | 280    | 148(52.86%) | 140     | 27(19.28%) |
| DMSO           | 282    | 155(54.96%) | 122     | 21(17.21%) |
| Fresh oocyte   | 120    |         |         | 61(50.83%) |

Different superscripts within columns indicates significance (P< 0.05)

4. Conclusion
Ethylene Glycol (EG) was the most suitable intracellular cryoprotectant for the cryopreservation of post-thaw in vitro matured goat oocytes.
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