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Short Communication

Effect of glutathione supplementation during sperm preparation and *in vitro* fertilization on ovine embryo development*

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ABSTRACT

The present study was conducted to investigate the effect of glutathione (GSH) supplementation during sperm preparation media (SPM) and *in vitro* fertilization (IVF) media on ovine embryo development. Six independent trials with a total of 273 oocytes were carried out with GSH supplementation at 0.3 mg / ml during SPM and IVF, 263 oocytes without supplementation formed the control. The mean cleavage rates were 48.46 ± 0.15 and 46.68 ± 0.12 per cent (P<0.01) in the treatment and control group, respectively. The percentage of morula (mean ± SE) that developed in treatment and control group were 24.58 ± 0.32 and 19.80 ± 0.24, respectively. The percentage of embryo that developed to the morula stage were significantly higher (P<0.01) in treatment group. GSH supplementation during SPM and IVF improved the cleavage and morula development.

Key words: Ovine, Glutathione, *In vitro* fertilization, Embryo.

The effect of growth factors, amino acids, antioxidants, energy sources, oxygen and temperature requirements have all been investigated in an attempt to improve *in vitro* embryo production rates. One area which has probably received less attention is that of the sperm preparation system. A major culture induced stress in IVF was enhanced oxidative damage, with increased reactive oxygen species (ROS) production (Orsi and Leese, 2001). Glutathione is the major non protein sulphydryl compound, which play an important role in protecting the cell from oxidative damage (de Matos *et. al.*, 1996). Glutathione supplementation during SPM and IVF act by protecting the sperm from free radical damage (Earl *et. al.*, 1997), sperm chromatin decondensation and male pronucleus formation (Yoshida *et. al.*, 1993) and reduction of nuclear disulfide bonds prior to exposure to the ooplasm (Perreault *et. al.*, 1984 and Yoshida *et. al.*, 1993). Hence the present study was conducted to determine the effect of GSH supplementation during SPM and IVF media on ovine embryo development.

All chemicals were purchased from Sigma Chemicals Company (St.Louis, MO, USA) and disposable plastic wares from Nunc (Denmark), unless otherwise stated.

Retrieval of oocytes

Oocytes were retrieved by slicing from the ovaries of ewes collected from slaughter house and graded as A, B, C according to Wahid *et. al.* (1992). Only A and B graded oocytes were used for the experiments.

In vitro maturation (IVM) of oocytes

A total of 536 A and B graded oocytes were matured using TCM 199 supplemented with 10 per cent fetal bovine serum at 38.5°C in an atmosphere of 5 per cent CO_2 incubator for 24 h. The maturation was assessed based on the

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expansion of the cumulus cells and extrusion of the first polar body.

***In vitro* fertilization of oocytes**

273 matured oocytes were subjected to six independent trials with GSH supplementation at 0.3 mg/ml in SPM and IVF, 263 oocytes without GSH supplementation formed the control. Epididymal sperms were collected and flushed into modified synthetic oviduct fluid (mSOF) containing 2.0 per cent sheep serum. The swim up method as described by Parrish *et. al.* (1995) was used to separate the motile sperms. The concentration of sperm was adjusted to 2 x 10^6 sperm / ml by diluting with mSOF medium. The matured oocytes were denuded from the cumulus attachment by vortexing for 90 sec and transferred to 75 ml droplets of mSOF medium at the rate of 15 oocytes per droplet for IVF. These droplets were inseminated with 2 ml of sperm suspension and co-incubated for 24 h at 38.5°C in 5 per cent CO_2 incubator.

***In vitro* culture (IVC)**

The oocytes were cultured in 50 ml droplets of mSOF for six days at 38.5° C in 5 per cent CO_2 incubator. The cleavage was assessed at 24 and 48 h post insemination and the developmental stages of cleaved embryos were monitored every 24 h up to 6 days. During the course of culture, the embryos were transferred to fresh mSOF medium every 48 h.

The result of the study revealed that in glutathione supplemented group out of 273 oocytes, 131 cleaved and 67 morula were obtained. In control group out of 263 oocytes, 124 cleaved and 51 morula were obtained. The mean cleavage rates were 48.46 ± 0.15 and 46.68 ± 0.12 per cent in the treatment and control group, respectively and the difference was significant ($P<0.01$). The percentage of morula developed in treatment and control group were 24.58 ± 0.32 and 19.80 ± 0.24, respectively. The percentage of embryo that developed to the morula stage were significantly higher ($P<0.01$) in treatment group when compared to the control. The significant difference ($P<0.01$) in the cleavage rate observed in the study disagreed with earlier reports of Luvoni *et. al.*, 1996 and Earl *et. al.*, 1997 who supplemented GSH during IVF and SPM respectively. GSH (L-r-glutamyl-L-cysteinyl-glycine) was a tripeptide major intracellular non protein sulphydryl, free thiol which protected cells against oxidation amino acid transport, protein synthesis and reduction of disulfides. The synthesis of GSH during oocyte maturation was a prerequisite for sperm chromatin decondensation and hence for male pronucleus formation after sperm penetration in mouse and hamster oocytes. Further sperm decondensation took place more slowly or incompletely in oocytes having insufficient GSH, resulting in asynchronous development of male and female pronucleus (Yoshida *et. al.*, 1993). The increased cleavage rate observed in our study due to the cumulative effect of GSH supplementation during sperm preparation media and IVF media. Moreover the improved cleavage rate and morula development observed due to the antioxidant effect of glutathione in protecting the sperm from free radical damage, sperm chromatin decondensation, oocyte activation and male pronucleus formation.

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