

COMPARISON OF TCM-199 AND SOF MEDIA ON IN VITRO DEVELOPMENT OF OVINE PREIMPLANTATION EMBRYOS*

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ABSTRACT

The aim of the present study was to compare the effectiveness of two different media viz undefined media (TCM-199) and semi defined (SOF) media for *in vitro* development of ovine preimplantation embryos. A total of 174 ovaries were sliced and 782 oocytes were recovered with a total recovery rate of 4.5 oocytes per ovary and cultureable oocytes recovery rate of 2.7 oocytes per ovary. The presumptive zygotes derived from *in vitro* maturation and fertilization (IVM/IVF) were randomly subjected for two *in vitro* culture (IVC) systems (TCM-199 and SOF). Statistical analysis revealed that highly significant ($P>0.01$) percentage of embryos reached morula after cleavage in SOF media (30.4 ± 1.14) than TCM-199 (21.7 ± 0.93). From this study, it was concluded that semi defined media like SOF support high rate of ovine morula development than undefined media like TCM-199 complex media due to amino acids included in SOF media and a biphasic effect of bovine calf serum inhibiting the first cleavage and promoting further morula and blastocyst development in TCM-199 media.

Key words: In vitro development, Ovine oocytes, TCM-199 and SOF media.

The *in vitro* embryo production technology has enabled mass embryo production for application in emerging technologies like cloning and transgenesis and for research on embryo physiology and metabolism. Of the steps in *in vitro* embryo production, *in vitro* culture is likely to have the largest effect on the development, since embryos are held in the media for eight days. There have been efforts for development of specific culture regimes capable of supporting preimplantation development of embryos as it is the most sensitive phase of development and has thus made it possible to obtain preimplantation embryos of all stages. The aim of the present study was to compare the effectiveness of two distinct media viz undefined

media and semi defined media for *in vitro* development of ovine preimplantation embryos.

MATERIALS AND METHODS

Collection of oocytes

Ovine ovaries were collected and transported in normal saline supplemented with 50 µg/ml gentamicin sulphate from a local abattoir to the laboratory with in half an hour of slaughter. Oocytes were recovered by slicing method and were pooled in oocyte washing media (Gordon, 1994). Oocytes were graded based on the number of layers of cumulus investment and the homogeneity of the ooplasm (Nandi *et al.*, 1998) and the oocytes with more than three layers of cumulus cells were selected for *in vitro* maturation.

In Vitro Maturation

The maturation medium was TCM-199 (Sigma M 5017) supplemented with 10 per cent fetal bovine serum (F 4135), sodium pyruvate 0.2mM (Sigma), gentamicin

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50 µg/ml (sigma), FSH 5 µg/ml (Follitropin V, Bioniche), LH 0.01 units /ml (Sigma L 5269), Oestradiol 17-β 1µg/ml (Sigma E 2758). The prepared media was filter sterilized and equilibrated in a CO₂ incubator for 2 h prior to use. The oocytes were washed three times in maturation medium and then placed in 50µl droplets (10 COCs each) of maturation medium which was overlaid with mineral oil in 60mm petridish (Nunc, Denmark). The oocytes were cultured for 24 h at 5 per cent CO₂ in air and at a temperature of 38.5°C.

***In Vitro* Fertilization**

Testis from adult rams were collected from a local abattoir and transported in normal saline supplemented with 50 µg/ml gentamicin sulphate. Ram sperm were collected from cauda epididymis and separated by the swim up method as described by Parrish *et al.* (1995). The suspension was further diluted with fertilization medium so as to get a final concentration of 2x10⁶ sperms /ml. After 24 h of maturation, oocytes were washed in fertilization medium and then transferred to 75 µl droplets of fertilization medium (15 oocytes/droplet). Sperm suspension at Hx 10³ were added to each fertilization droplets containing oocytes and co incubated for 24 h at 5 per cent CO₂ and 38.5°C.

***In Vitro* Culture**

The presumptive zygotes were randomly subjected to two different *in vitro* culture systems (TCM-199 with 10 per cent FBS and SOF). The presumptive zygotes were washed and placed in the preequilibrated IVC droplets. On alternate days the IVC medium was replaced with fresh medium. Data from 8 trials were analysed by student's t test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

A total of 174 ovaries were sliced and 782 oocytes were recovered with a total recovery rate of 4.5 oocytes per ovary and cultureable oocytes (477) with recovery rate of 2.7 oocytes per ovary. The cleavage rate and percentage of embryos that reached morula in TCM-199 and SOF media were 44.6± 0.43, 21.7±0.14 and 47.7± 5.22, 30.4±0.25 respectively. There was no significant difference (P>0.05) in the cleavage rates and

the percentage of morula developed was highly significant (P>0.01) in the two media. Gardner (1994) suggested that SOF support high rate of sheep blastocyst development and hatching and cell number was equivalent to those developed *in vivo*. This might be due to amino acids included in the medium. Pinyopummintr and Bavister (1991) emphasized a biphasic effect of bovine calf serum inhibiting the first cleavage and promoting further morula and blastocyst development. In sheep premature blastulation has been reported for embryos cultured in the presence of human serum. However, the role played by serum in embryo development *in vitro* was largely unknown. Moreover the composition of bovine sera was highly variable from batch to batch (Price and Gregory, 1982) and some batches of serum were ineffective or can even be toxic to embryos (Bavister, 1995). Moreover the unknown environment of undefined media would make it difficult if not impossible to examine effects of specific components of culture medium on embryo development. Serum also has the effect of masking problems in the culture system and can buffer the embryo from less than optimal environmental conditions (Gandhi *et al.*, 2000). However, the semi defined media SOF would avoid risk of contamination and would allow researchers to examine more accurately the requirements of embryos. From this study, it was concluded that semi defined media like SOF support high rate of ovine morula development than undefined complex media like TCM-199. The bovine calf serum added in TCM-199 had a biphasic effect inhibiting first cleavage and further morula and blastocyst development.

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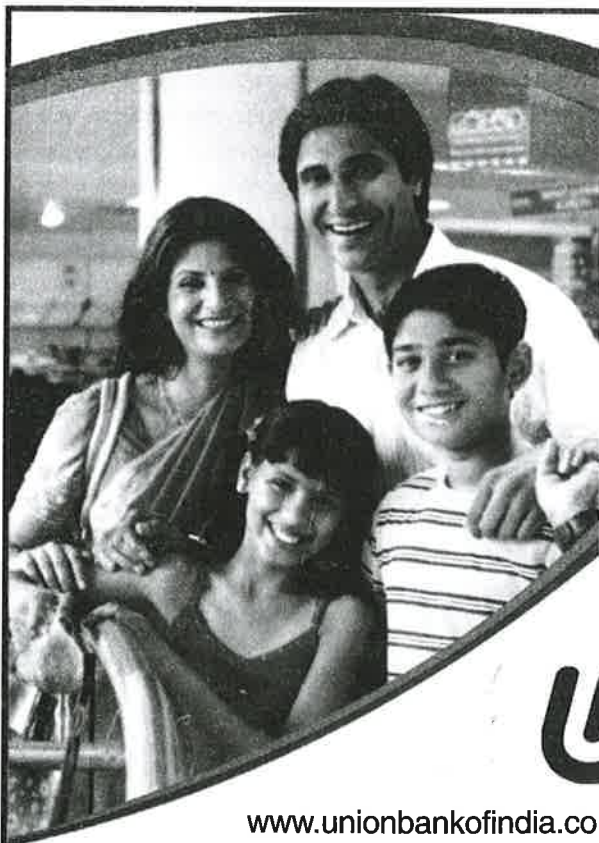
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