EFFECTS OF DIFFERENT MATURATION AND CULTURE MEDIA ON IVF OF SHEEP OOCYTES

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ABSTRACT

This study was performed 1) to compare different ratios of foetal calf serum (FCS) for *in vitro* maturation (IVM) of sheep oocytes and 2) to investigate different culture media and supplements for ovine embryo production *in vitro*. Primary oocytes collected from ovaries of slaughtered Kivircik ewes were divided into 2 groups randomly and incubated *in vitro* for 26 hours at 39°C in a humidified atmosphere of 5% CO_2 in air, TCM 199 medium supplemented with 10µg/ml follicle stimulating hormone, 10µg/ml luteinizing hormone, 1µg/mlestradiol 17β and 10% FCS (low) or 20% FCS (high) was used as maturation medium. After *in vitro* maturation and fertilization, presumptive zygotes were again divided into 2 groups randomly, and co-cultured for 6 days with sheep oviductal epithelial cells. In the first culture group (TCM), TCM 199 medium supplemented with 55 sheep estrous serum (SES), and in the second group synthetic oviduct fluid (SOF) medium supplemented with 20% SES were used. The cleavage and morula rates among groups (low +TCM, high+TCM, Low+SOF, High+SOF) were 38.8^{ab}, (38/98), 33.3^{b}, (28/84), 52.0^{a} (53/102) and 53.0^{a} (44/83); and 22.4^{ab} (22/98), 16.7^{b} (14/84), 31.4^{a} (32/102) and $34.9\%^{a}$ (29/83), respectively. Differences among groups with different letters (a,b) were important statistically ($p<0.05$). The results of this study showed that *in vitro* culture (IVC) of sheep embryos derived *in vitro* could be better in SOF medium than TCM 199.

Key words: Sheep, IVM, Serum supplementation, TCM 199.

INTRODUCTION

The importance of reproductive techniques on animal genetic improvements and breeding is increasing. For the production of large numbers of embryos from slaughtered ewes, there are different *in vitro* maturation, fertilization and culture systems (Walker *et al.*, 1992; Walker *et al.*, 1994; O'Brien *et al.*, 1994; Gomez *et al.*, 1998). In these systems, tissue culture medium (TCM 199) supplemented with hormones has been used usually for *in vitro* maturation of sheep oocytes obtained from ovaries of slaughtered ewes (O'Brien *et al.*, 1994; Byrd *et al.*, 1997; Birler *et al.*, 1999a)

For culture or co-culture of *in vitro* fertilized oocytes, TCM 199 or SOF medium (Walker *et al.*, 1992; Walker *et al.*, 1994; Byrd *et al.* 1997; Gomez *et al.*, 1998) supplemented with FCS (Tervit *et al.*, 1972) or SES (Madan *et al.*, 1992; Walker *et al.*, 1992; Holm *et al.*, 1994; Walker *et al.*, 1994), or sometimes without supplementation (O'Brien *et al.*, 1994; Gomez *et al.*, 1998) have been used. Sheep oviductal epithelial cells (SOEC: Holm *et al.*, 1994; Birler *et al.*, 1999), bovine oviductal cells (BOEC: Byrd *et al.*, 1997) and cumulus cell monolayer (Madan *et al.*, 1992) have also been used for co-culture with *in vitro* fertilized embryos.

This study was performed 1) to compare different ratios of FCS for IVM of sheep oocytes and 2) to investigate different culture systems for ovine embryo production *in vitro*, in a 2 X 2 factorial design.

MATERIALS AND METHODS

In this study, primary oocytes collected by slicing ovaries of Kivircik ewes slaughtered in a local abattoir in Istanbul were used. The ovaries were brought to the laboratory within 2 hours of slaughter in a vacuum flask containing 0.9% sodium chloride (NaCl) at 30-35°C. Ovaries were sliced and washed with 0.9% NaCl supplemented with 1% FCS in a watch glass. Oocytes with a homogenous cytoplasm and compact cumulus cells were selected under stereo microscope and after washing 0.9% NaCl supplemented with 10% FCS, were divided into 2 maturation groups randomly and matured at 39°C under humidified 5% CO_2 in air for 26 hours.

Group 1 (low): TCM medium supplemented with 10% FCS, 10 µg/ml follicle stimulating hormone (FSH), 10µg/ml luteinizing hormone (LH) and 1µg/ml estradiol 17 β was used.

Group 2 (High): TCM medium supplemented with 20% FCS, 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17 β was used.

After maturation, oocytes were transferred to fertilization medium supplemented with 20% SES and inseminated for 18 hours with fresh ram semen washed with BSA and heparin added synthetic oviduct fluid (SOF) medium. The final sperm concentration was 1 X 10^6/ml.

After fertilization, presumptive zygotes were again divided into 2 groups and co-cultured with sheep oviductal epithelial cells (SOECs) for 6-7 days.

In the first culture group (TCM), TCM medium supplenmented with 5% SES and in the second culture group, SOF medium supplemented with 20% Ses were used. The final four experimental groups of this study are presented in Table 1.

Table 1: Experimental groups

Culture Groups	Maturation Groups	
	Low Group	High group
TCM	Low+TCM (n=98)	High+TCM(n=84)
SOF	Low+SOF(n=102)	High+SOF(n=83)

SOECs were collected by washing of sheep oviducts at the same time with oocytes collection and after washing twice in 0.9% NaCl with 10% FCS, and once in TCM medium supplemented with 10% FCS, cultured at 39°C under 5% CO_2 in air until co-culture with presumptive zygotes.

Data were analyzed using Chi-square analysis and Student's t-test.

RESULTS AND DISCUSSION

According to the group (Low+TCM, High+TCM, Low+SOF, High+SOF), cleaved oocytes were 38.8 (38/98), 33.3 (28/84), 52.0 (53/102) and 53.0 (44/83), and oocytes reached to morula stage were 22.4 (22/98), 16.7 (14/84), 31.4 (32/102) and 34.9% (29/83), respectively (Table 2).

Although irrespective of culture groups, cleavage and morula rates between maturation groups were not different (Fig. 1), irrespective maturation groups, cleavage and morula rates between culture groups were significantly different ($p<0.01$) (Fig. 2). Morula rates obtained in this study are similar with the findings of Holm *et al.* (1994) and Gomez *et al.* (1998) but all morula were arrested at that stage.

Byrd *et al.* (1997) and Sevillano *et al.* (1997) reported low blastocyst rates, and Naqvi *et al.* (1992) reported very low blastocyst rates. However, Holm *et al.* (1994) who fertilized and culpured sheep oocytes in SOF medium with 20% SES under 5% O_2, 5% CO_2 in

Table 2: Number of oocytes cleaved and reached to morula stage, after co-culture for 6 days.

Groups	N.of oocytes	N.of cleaved (%)	N.of morula (%)	% of morula/ cleaved
Low+TCM	98	38(38.8)^{ab}	22(22.4)^{ab}	57.9
High+TCM	84	28(33.3)^{b}	14(16.7)^{b}	50.0
Low+SOF	102	53(52.0)^{a}	32(31.4)^{a}	60.4
High+SOF	83	44(53.0)^{a}	29(34.9)^{a}	65.9

^{a,b} Values with different superscripts within the same column are different ($P<0.05$).

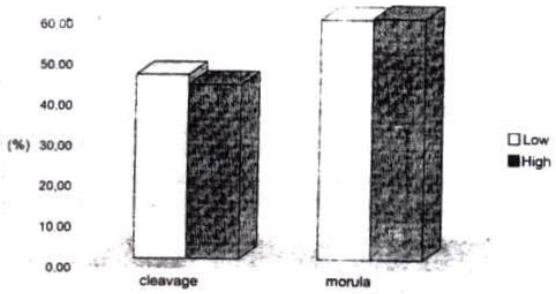

Fig. 1: Cleavage and morula (from cleaved embryos) rates between maturation groups.

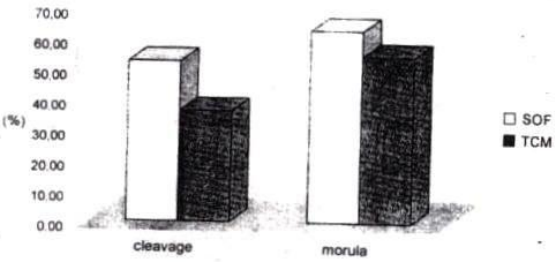

Fig. 2: Cleavage and morula (from cleaved embryos) rates between culture groups.

90% N_2, reported high blastocyst rates like Walker *et. al.* (1994) and O'Brien *et al.* (1997). There are many possible causes of arresting at morula stage, such as culture mediua composition, oxygen tension during culture period and embryo abnormalities. Tervit *et al.* (1972) suggested that embryo culture medium and especially oxgen tension are very important for embryo development.

In conclusion, results of this study demonstrate that sheep oocytes derived *in vitro* could be developed better in SOF medium than TCM, and SOF medium improves the rates of cleavage and morula. Second conclusion from these results is that low ratio of FCS can be sufficient for oocytes maturation and subsequent embryo development.

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