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EFFECT OF SUPPLEMENTATION OF OVINE OVIDUCTAL CELLS WITH LH AND OESTRADIOL-17β ON EMBRYO DEVELOPMENT

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In sheep, towards the end of follicular phase of the oestrus cycle, plasma concentration of E_2 increases and this increase is followed by an increase in LH secretion. The secretion of E_2 and LH can affect the secretary function of the oviductal cells and hence facilitate embryonic development *in vitro*. Therefore, the aim of this study was to measure the effect of supplementing the oviductal cells with Luteinizing Hormone (LH) and oestradiol - 17β (E_2) on embryo development.

Materials and Methods

Collection of ovaries and occyte aspiration : Ovaries were collected from slaughtered Karaman and Merino sheep. The ovaries were placed in TCM199/HEPES medium (Sigma; M-7528) supplemented with 10% inactivated sheep serum (Sigma; S-2263), 50 µg/ml heparin (Sigma; H-3149), 100µg/ml antibiotic mixture (penicillin G; Sigma; P-7794 and streptomycin sulphate, Sigma; S-0890) and brought to laboratory within two hours at 37°C. Oocytes, from 3-8 mm follicles, were aspirated by vacuum.

Preparation of oviductal cells : Both oviducts were collected from 6-8 animals. The cells from ampulle oviduct were physically removed and pooled. The cell concentration was adjusted to 4 x 10^6 cell/ml and cultured in TCM 199 / HEPES supplemented with 5µg/ml LH (Sigma; L-5269), 5µg/ml oestradiol-17β (Sigma; E-1024), 100 µg/ml antibiotic mixtuer and 10% heat inactivated normal sheep serum.

Oocyte maturation : To avoid effect of metabolic contamination, the oocytes were cultured in 3ml of TCM199/HEPES supplemented with 3µg/ml LH, 3µg/ml FSH (Sigma; F-8174), 4µg/ml E2 10% (v/v), heat inactivated sheep serum and 100 µg/ml antibiotic mixture for 24 hours.

Sperm capacitation : Sper-matozoa were obtained from the caudal epidiydiymis of slaughtered Karaman and Merino rams. Spermatozoa from 3 different rams was pooled and taken to the lab at room temperature within two hours and cultured in TCM199/HEPES supplemented with 62.5 µg/ml heparin (sigma; H-3149), 150µg/ml BSA (Sigma; A-2058), 100µg/ml CAI (Sigma; C-5149) and 100mg/ml antibiotic mixture for 50-60 minutes in a mummified atmosphere of 5% CO_2 in air at 37°C. For fertilization, a volume of the media containing 6x10^5 forward moving spermatozoa added into the 3 ml of fertilization medium TCM 199/HEPES supplemented with 10µg/ml hypotaurin (Sigma; H-1384), 2 µg/ml heparin, 4 µg/ml BSA and 100 µg/ml antibiotic mixture and incubated for 24 hours.

Embryo culture : The presumptive zygotes were transfered into TCM 199/

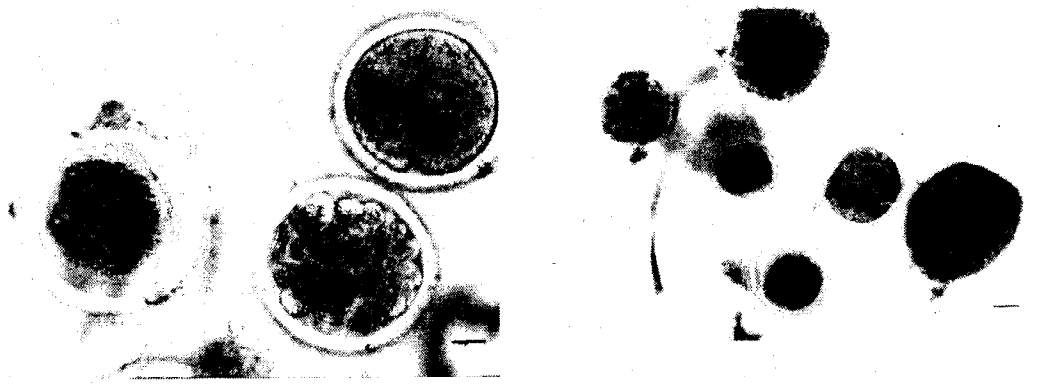

Figure 1. Photomicrograph of sheep embryos developed in culture. On the left panel, embryos developed after the addition of LH and oestradiol - 17β, while embryos developed after the supplementation of LH and E2 plus oviductal cells have been shown on right panel. **B**, blastocel; **BL**, blastocyst; **BPH**, Blastocyst preparing to hatch; **HB**; hatched blastocyst; **M**, morulae; **ICM**, inner cell mass; **Z**, Zona pellucida, **T**, trophectoderm. Scale bar = 100mm.

Table 1 : The number of oocytes cultured and developed to morulae, blastocyst and hatched stages.

Groups	Number of oocytes cultured	96 h after sperm addition	144h after sperm addition	168h after sperm addition
		Morulae (%)	Blastocyst (%)	Hatched (%)
Control	138	23.2 (32/138)	8.6 (12/138)	2.8 (4/138)
LH + E_2	138	36.2 (50/138)*	16.7 (23/138)*	5.1 (7/138)*
LH + E_2 + Ov. Cells	138	52.2 (72/138)**	28.3 (39/138)**	11.6 (16/138)**

* $P < 0.05$ as compared with Control ** $P < 0.05$ as compared with two groups.

HEPES supplemented with 0.55 µg/ml sodium pyruvate (Sigma; P-4562), 100µg/ml L-Glutamine (Sigma; G7513), 4µg/ml BSA, 100 µl/ml heat inactivated sheep serum, and 100 µg/ml antibiotic mixture for 6 days. Every 48 hours the old media was renewed. The zygotes were cultured in this media and kept as control, whereas the others cultured in the same media containing 5µg/ml LH, 5µg/ml E2 or 5µg/ml LH, 5µg/ml E2 with oviductal cell were kept as test groups. Three days after the beginning of the embryo culture, plates were examined under a light microscope and the number of embryos at morulae satages (16-64 cells) counted at 200 X magnifications. Blastocytes were differentiated by blastocoeal, inner cell mass and trophoblast. Only blastocytes with fully formed cavity were counted. Hatched embryos were differentiated by absence of zona, presence of thick trophectoderm at 400 x magnifications.

Statistical analysis : Data were analysed by using chi-square test.

Results and Discussion

The addition of LH and oestradiol - 17β to the medium had positive effect on the development of embryos to morulae, blastocyte and hatched blastocyte. There were 11.2%, 8.1% and 2.3% increases in the number of embryos at morulae, blastocyst and hatching stages over the

control group ($P < 0.05$). Presumptive zygotes cultured in media supplemented with oviductal cells, LH and oestradiol - 17β had significant increases in embryo development over the other two groups ($P < 0.05$, Table 1, Figure 1).

Addition of LH and oestradiol significantly increased the number of embryos at morulae, blastocyst and hatched stages as compared with control group. Gandolfi and Moor (1987) have examined the effects of oviductal cell support on the cleavage and viability of fertilized sheep eggs and reported 95% and 42% of cultured zygotes developed to morulae and blastocyst stages respectively. Using the same culture medium, the beneficial effect of oviductal cells on blastocysts formation has also been reported by Li *et al.*, (2000). In another experiment, oocytes matured and fertilized *in vitro* and co-cultured for 5 days with sheep oviductal epithelial cells, 43% cultured oocytes developed to the morulae stage and 38% of cultured reached blastocyst stage (Czlonkowska *et al.*, 1992). In this study, 52% of cultured eggs developed to morulae stage by the addition of LH and oestradiol to the culture of presumptive zygotes and oviductal cells. By using TCM-199, Bernardi and Delouis (1995) cultured sheep zygotes with oviductal cells for 8 days and they reported that 22.9% of cultured zygotes reached blastocyst stages. In this study, 28.3% of cultured eggs became blastocyst when cultured with oviductal cells supplemented with LH and oestradiol. Thus, supplementing the oviductal cells with LH and oestradiol has positive effect. The reason for this might be because E_2 and LH increased the oviductal secretion of IGF-I, II or IGF binding proteins and thereby enhanced embryo development.

Summary

The aim of this research was to study the effect of supplementing the oviductal cells with Luteinizing hormone (LH) and Oestradiol - 17β (E_2) on sheep embryo development *in vitro*. Addition of 5μg/ml LH, 5μg/ml E_2 to embryo culture significantly increased the percentage of embryos over the control group. Addition of LH and E_2 to the culture of zygotes containing oviductal cells, caused further significant increases in the percentages embryos at morulae, blastocyst and hatched stages over the control and LH-E_2 added group by 28.75, 19.88 8.89% and 15.58, 11.91, 6.39% respectively.

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