

be specific for the round spermatid using anti-goat rabbit transition protein (TP2) antibody.

Results: Viability of the cells cultured by monolayer method, organ culture method or in a biodegradable scaffold was 60%, 70% and 95% respectively. Microscopic observation indicated that 80% of the recovered germ cells developed to late spermatids and 20% reached elongated spermatids. In contrast, only 20% of germ cells cultured by the organ culture method developed to round spermatids. Monolayer culture method showed that only 10% of cultured germ cells developed to round spermatids. No elongated spermatids was found in cells cultured either by organ culture method or by monolayer method.

Conclusions: A culture system consisting of a biodegradable scaffold could support the in vitro differentiation of mouse male germ cells. Compared to the conventional monolayer culture method and organ culture method, the system appeared to be superior.

O-15 Effects of IGF-I, TGF- α and LIF on Apoptosis of Blastomere and oct4 Gene Expression in Mouse Preimplantation Embryos

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Objective: It has been shown that insulin-growth factor-I (IGF-I) and transforming growth factor- α (TGF- α) are important inhibitors of cell death and promote preimplantation embryo development, and leukemia inhibitory factor (LIF) is implicated in inhibiting the differentiation of cells. Oct4 is a transcription factor that maintains totipotency of the cells and its effects were recently reported to be dependent on its concentration in the nucleus. Especially, oct4 mRNA and protein are predominantly found in the inner cell mass (ICM) of the blastocysts. This study was carried out to investigate the effects of IGF-I, TGF- α and LIF on oct4 expression, in relation to cell number and apoptosis in mouse preimplantation embryos.

Materials and Methods: 2-cell embryos were collected post-hCG 48 hr and were cultured for 72 hr (control). Treatments consisted of following groups, each of which was cultured with IGF-I (1.7 nM), TGF- α (250 pM) and LIF (1000 unit/ml). Semi-quantitative RT-PCR was used to assess the gene expression of oct4 and cell apoptosis was detected by TUNEL counterstained with hematoxylin.

Results and Discussion: Oct4 gene expression was similar in all groups at 4-cell and morula stage, but increased in the IGF-I and TGF- α treated groups at blastocysts stage. The total number of blastomere was not different among all groups. But, the proportion of TUNEL-labeled nuclei in blastocysts significantly decreased from 28.7% (control) to 18.3% (IGF-I) and 10.8% (TGF- α), respectively. Taken together, in blastocysts, the anti-apoptotic action of IGF-I and TGF- α increased the ICM cell number combined with reduced cell death, therefore, oct4 gene expression increased. It is suggested that amount of oct4 gene expression may be associated with blastocyst quality. This study might be applicable to improve the culture system of the blastocyst transfer in human ART.