

# Co-culture of Mouse Two-cell Embryos on Human Endometrial Stromal Cells in Proliferative and Secretory Phases

Co-cultivo de Dos Células Embrionarias de Ratón sobre Células Estromales Endometriales Humanos en Fases Proliferativa y Secretora

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FAKHERI, T.; NANKALI, A.; KHAZAEI, M.; GHANBARI, A.; ABEDINI, M. & SAEIDIBOROJENI, H. Co-culture of mouse two-cell embryos on human endometrial stromal cells in proliferative and Secretory phases. *Int. J. Morphol.*, 30(4):1338-1342, 2012.

**SUMMARY:** There were no significant differences in the distribution of embryos reaching to 2- cells, 4- cells, morula or blastocysts culturing on human endometrial stromal cells (Secretory or proliferative phases). The percent of morula in stage A (without fragmentation), stage B (<25% fragmentation), stage C (25-50% fragmentation) and stage D (>50% fragmentation) and did not showed significant differences between two coculture groups. Thus, the phase that the endometrial stromal cells were in thereby did not affect on the quality of embryos.

**KEY WORDS:** Co-culture; Embryo; Endometrium; Mice; Proliferative phase; Secretory phase.

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## INTRODUCTION

Treatment of infertile couples has been progressed by developing *in vitro* fertilization (IVF) that preimplantation embryos are routinely transferred into the uterus.

The concept of improved human preimplantation development and implantation ability by co-culturing embryos in the presence of another cell type (feeder cells) has led to the development of the co-culture system (Beydoun *et al.*, 2010; Teklenburg & Macklon, 2009).

Multiple cell types have been used for this purpose, ranging from human reproductive tissues, such as oviducts (Bongso *et al.*, 1994; Yeung *et al.*, 1992), endometrium (Jayot *et al.*, 1995), oviduct-endometrial sequential co culture (Bongso *et al.*, 1994), and cumulus-granulosa cells (Freeman *et al.*, 1995; Saito *et al.*, 1994), to nonhuman cells (Wiemer *et al.*, 1993) or nonhuman cell lines (Menezo *et al.*, 1990; Schillaci *et al.*, 1994) and even cells from ovarian carcinoma (Ben-Chetrit *et al.*, 1996). Unfortunately, there is no general agreement on the efficacy of different co culture systems (Bavister, 1995); not even has the utility of the co culture itself

compared to that of a chemically defined medium been proven (Bavister; Tucker *et al.*, 1996). Even with the most extended co culture system, i.e. Vero cells, results in randomized studies are discrepant (Sakkas *et al.*, 1995). Moreover, endometrial thickness of donor patient is in correlation with the rate of positive result of IVF. In other words, if the endometrium be in the secretory phase, the implantation of transplanted embryo is higher than that for proliferative phases (Al-Ghamdi *et al.*, 2008; Richter *et al.*, 2007).

The better results of IVF achieved in secretory phase endometrium, attracted many researchers to add hormones of this phase to the media of co-culture system. The results of these studies did not show improvement in the rate of the implantation and delivery. But, neither the studies which used co-culture alone nor the ones added the hormones secreted during Secretory phase endometrium distinguished the phase of endometrium used by. The aim of this study is to determine if there is any effect of endometrial phase on the development of the embryos that are co cultured with endometrial stromal cells.

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## MATERIAL AND METHOD

This study was carried out in Fertility and infertility research center, Kermanshah University of Medical Sciences. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences.

Endometrial biopsies were obtained from reproductive fertile women (20–37yr). With normal menstrual cycles who were undergone bilateral tubal ligation or Anterior – posterior colporrhaphy with informed consent forms. The sample collection was done following filling the acceptance form approved by research council of Kermanshah University of Medical Sciences, Iran. According the menstrual phase, the samples divided into two proliferative or Secretary.

Endometrial tissues were collected separately by the groups in 50 ml conical tubes containing phosphate buffered saline (PBS), supplemented with 100 IU penicillin and 100 mg/ml streptomycin, and then cooled on crushed ice in a thermos flask and immediately transported to the laboratory.

**Endometrial culture.** Endometrial samples were minced into small pieces of less than 1mm and subjected to mild collagenase digestion. Endometrial stromal cells were isolated as previously described. The stromal cells were cultured and grown to confluence in steroid-depleted medium; DMEM/F12 and 25% MCDB-105 (Sigma Chemical Co.) containing antibiotics, supplemented with 10% FBS and 5 mg/mL insulin (Sigma Chemical Co.). The homogeneity of cultures was determined by morphological characteristics.

**Mouse embryo assay.** Two-cell embryos were flushed from the oviducts on day 2 of pregnancy from 8-week-old Balb/c mice. Following intraperitoneal injection of PMSG (7.5 unit) that followed by 5 unit injection of HMG (48 hr later) the embryos were collected from super ovulated mice. The appearance of vaginal plaque demonstrated the zero day of pregnancy. The embryos co-cultured with the same medium (DMEM/F12, 10% FBS, 5 mg/mL insulin) in the presence of endometrial stromal cells obtained from either secretory or proliferative phase. Cultured embryos were observed every 72 hr by invert microscope. The progress in the growth and develop of the embryos evaluated during 4-8 cell and blastocyst stages.

**Data analysis.** The microscopic data of co cultured embryos of two experimental groups were evaluated by using Spss software for windows (Version 16). This data included, the number of morula and Blastocysts that evaluated with T- Test analysis where the quality of zona pelucida and the ratio of granulation with c2 ( $P < 0.05$ ).

## RESULTS

Two hundred mouse embryos that cocultured in two conditions; with endometrial stromal cells in proliferative and secretory phases were monitored microscopically during cultivation period (Fig. 1). The data showed there were not significant differences in the distribution of embryos reaching to 2- cells, 4- cells, morula or blastocysts of two groups (Fig. 2).

The quality of embryos in morula stage was evaluated according to the degree of fragmentation and granulation, appearance of zona pelucida and blastomers in four classifications. The data showed, although the percent of morulas in stage B (<25% fragmentation) was higher for secretory group but it was lower in stage A (without fragmentation) in compared with proliferative group. For other two low quality stages, the percent of morulas in stage D (>50% fragmentation) and stage C (25-50% fragmentation) did not showed significant differences (Fig. 3). Thus, the phase that the endometrial stromal cells were in thereby did not affect the quality of embryos.

## DISCUSSION

The present study showed that not only the number of two-cell mouse embryos reaching four-cell morula in two different co-culture media (consisting proliferative and secretory stromal cells) but also the quality of blastomers in morula stage did not differ statistically in two different co-culture groups. However, minor differences only seen in 4 quality of staged embryos. Data from a number of studies provide convincing evidence of a chemical dialogue between the developing embryo and the maternal endometrium (Edwards, 1995). This embryonic- endometrial cross-talk may be beneficial not only to improve the blastocyst rate, but most importantly for the activation of specific paracrine molecules in a timely manner that may improve the chances of implantation of the embryo (Tazuke & Giudice, 1996). Recently, it has demonstrated that a co-culture system with human endometrial epithelial cells (EEC) is beneficial to the human blastocyst because of the induction of secretion of embryonic paracrine molecules, absorption of toxic material and altering in metabolism involving in the media (De los Santos *et al.*, 1995). Moreover, exogenous treatment of endometrial cells with progesterone or progesterone plus estradiol did not improve the development embryo in a co-culture system (Goff & Smith, 1998).

In conclusion, the data of this study indicated that the endogenous steroids from secretory endometrial stromal cells did not affect on the development embryo as a paracrine process.

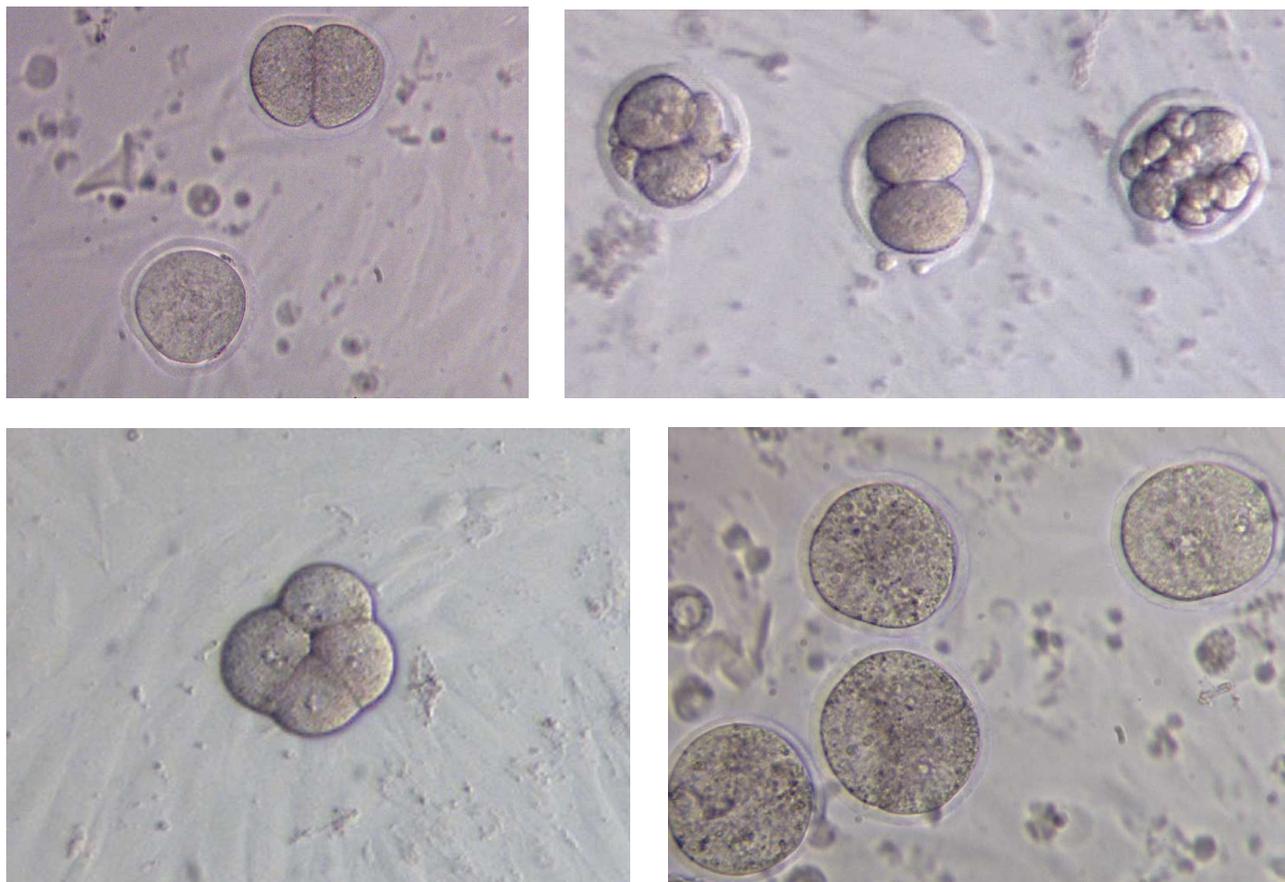


Fig. 1. Developmental stages of mouse embryos observed under light microscope. A. Unfertilized oocyte and two staged embryo on proliferative staged endometrial stromal cells (40X), B. Unfertilized oocyte and two staged embryo on secretory staged endometrial stromal cells (40X), C. Four staged embryo on proliferative staged endometrial stromal cells; initiation of compaction (10 X), D. Four staged embryo on secretory staged endometrial stromal cells; compaction (40X).

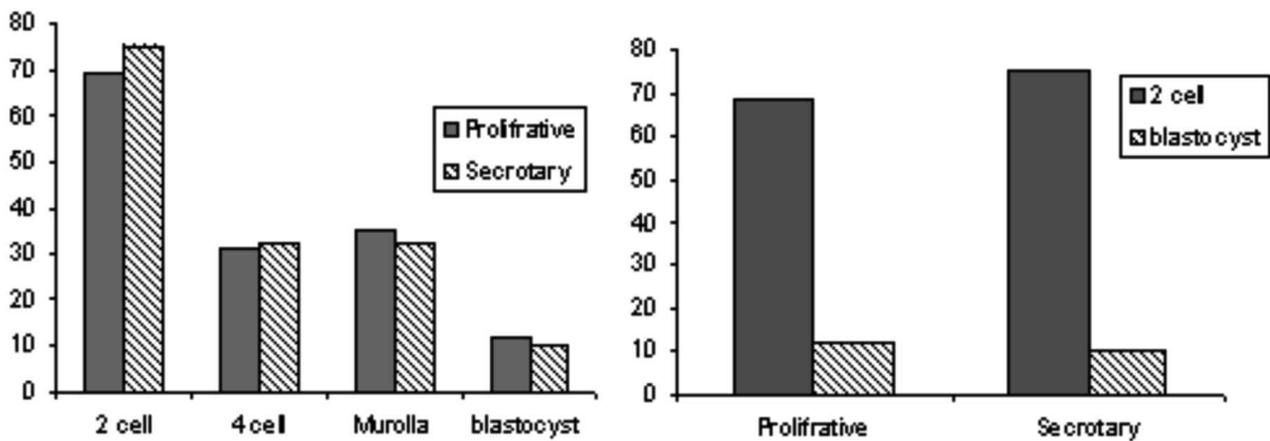


Fig. 2. A. The distribution of staged mouse embryos (2 cells, 4 cells, morula and blastocyst). B. Comparison the distribution of blastocysts and 2 cells mouse embryos in two co-culture feeder systems of this study.

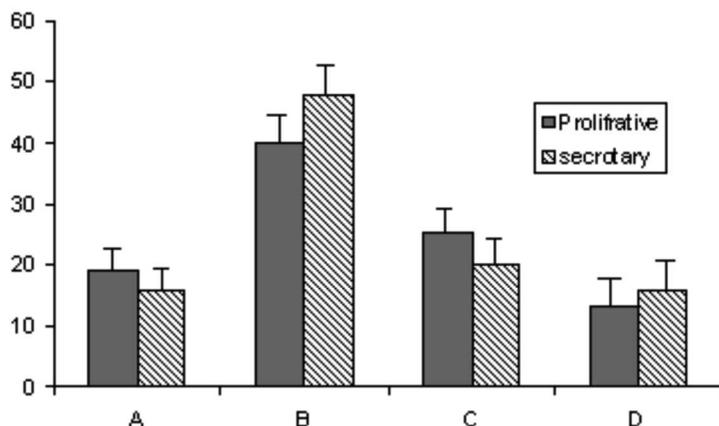


Fig. 3. The distribution of mouse embryos in 4 staged qualities according two coculture system of this study. A. Without fragmentation. B. 25% fragmentation. C. 25-50% fragmentation, D. >50% fragmentation (\* $P < 0.05$ ).

## ACKNOWLEDGEMENTS

The authors would like to express their deep gratitude to the Office of the Vice Chancellor for research of Kermanshah University of Medical Sciences and staff of Fertility and infertility research center for helpful assistance. This research was supported by a research grant (no U-477) from Kermanshah University of Medical Sciences.

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**RESUMEN:** No hubo diferencias significativas en la distribución de los embriones en los cultivos que llegan a las 2 y 4 células, mórula o blastocistos sobre las células del estroma endometrial (fases proliferativa y secretora). El porcentaje de mórulas en etapa A (sin fragmentación), etapa B (<25% fragmentación), etapa C (25-50% de fragmentación) y etapa D (>50% fragmentación), y no mostraron diferencias significativas entre los dos grupos de co-cultivo. Así, la fase en la que se encontraban las células estromales endometriales no afectaron la calidad de los embriones.

**PALABRAS CLAVE:** Co-cultivo; Embrión; Endometrio; Ratones; Fase proliferativa; Fase secretora.

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Received: 01-03-2012

Accepted: 08-05-2012