Co-culture techniques in assisted reproduction: history, advances and the future

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Abstract

The co-culture technique was first implemented in animal models in the mid-sixties in an attempt to improve the suboptimal conditions provided by culture media. Since then, autologous endometrial co-culture was introduced in human IVF, and different outcomes were reported regarding blastulation, fragmentation and implantation rates. Various growth factors and cytokines were proposed to be involved in the mechanism by which co-culture systems improved embryo development. However, randomized controlled trials are still warranted to provide evidence on the efficacy of endometrial co-cultures in the era of modern sequential- and mono-culture media.

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Introduction

The culture of mammalian embryos from one-cell to the expanded blastocyst stage is an inefficient process: on average about 50% of long-term cultured embryos cleave regularly and develop to the blastocyst stage, depending on the maternal age, in a good IVF laboratory and under standard culture conditions. From this minority, almost half fail to develop further when placed in the endometrium. The increased viability of certain cells, cultured in the presence of a feeder layer of another cell type has been documented in numerous *in vitro* culture systems (Köhler and Milstein, 1975; Martin, 1981). In an attempt to improve the suboptimal conditions present during in vitro culture, Cole and Paul co-cultured 2-cells murine embryos on an immortalized feeder cell line (irradiated HeLa cells, a cell line derived from cervical cancer cells taken from Henrietta Lacks) through the hatching blastocyst stage in 1965, achieving increased blastulation rates, when compared with embryos cultured in medium alone (Cole et al., 1965). Four-cell porcine embryos were also shown to have improved development when co-cultured on porcine endometrial cell monolayers (Allen and Wright, 1984). Bovine uterine fibroblast monolayers were also reported to support increased hatching of bovine embryos (Kuzan and Wright, 1982). Therefore, as results from these early studies suggested that perhaps uterine/ovarian fibroblasts appear to provide the same contribution for the support of embryo viability as endometrial cells, and no direct comparative studies were available the specificity of the cell type used as the feeder monolayer presented an intriguing question and cell contact was inferred as being responsible for the enhanced *in vitro* embryonic development observed in both cases.

The co-culturing technique has also been utilized in human IVF. Beginning in 1989, Wiemer et al. compared outcomes of conventional media with co-culture of human embryos and fetal bovine uterine fibroblasts. They obtained better results in the co-culture

group in respect to embryo morphology, implantation and ongoing pregnancy rates. They defined "the most outstanding morphological characteristic of co-cultured embryos" as "the expanded appearance of their blastomeres" (Wiemer et al., 1989). Ampullary cells of human origin have also been used (Bongso et al., 1989). Oviductal tissue was obtained from fertile women undergoing hysterectomy. Cleavage, cavitation, blastulation and hatching rates were found to be higher in the co-culture group when compared with controls (with patient's serum alone). Moreover, fragmentation and unequal cleavage were significantly reduced. It was therefore clear that human ampullary cells support human embryonic development (Bongso et al., 1989). A great enthusiasm arose then in the beginning of the 90's that co-culturing human embryos on feeder cells, of human or non-human origin, could supply essential requirements for the growing preimplantation embryos, promoting improved pregnancy rates.

Consequently, in order to promote human embryo development in vitro, various non-human cell lines have been used for co-culture, eg. bovine uterine fibroblasts (Wiemer et al., 1989), bovine oviductal epithelial cells (Bongso et al., 1989; Bongso et al., 1991; Bongso et al., 1992; Wiemer et al., 1993; Wiemer et al., 1994; Morgan et al., 1995; Tucker et al., 1995; Tucker et al., 1996; Vlad et al., 1996; Wiemer et al., 1996; Yeung et al., 1996; Ando et al., 2000) and Vero cells (Menezo et al., 1990; Menezo et al., 1992; Van Blerkom 1993; Sakkas et al., 1994; Schillaci et al., 1994; Kaufman et al., 1995; Magli et al., 1995; Turner et al., 1996; Guerin et al., 1997; Veiga et al., 1999; d'Estaing 2001). The Vero lineage was isolated from kidney epithelial cells extracted from an African green monkey (Menezo et al., 1990). Vero cells are also commonly used for vaccine production, and their selection for co-culture stems from a common embryologic origin of kidneys and of the genital tract. In this study, the blastocyst formation rate was found to be higher in the co-culture group and expanding and hatching blastocyst number was increased.

In a study by Desai and Goldfarb (1998), the pattern of growth factors and cytokine release by Vero cells was analyzed. Enzyme-linked immunoassay (ELISA) was applied to investigate the concentrations of platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ), interleukin-6 (IL6), leukemia inhibitory factor (LIF) and epidermal growth factor (EGF) secreted by Vero cells in co-culture conditions. A considerable patient-to-patient variation was assessed between the 11 patients enrolled in the study. This report has underlined one drawback of co-culture technique, as an exposure of the preimplantation embryo to a highly variable and unpredictable milieu of growth factors and cytokines (Desai and Goldfarb, 1998). However these data also supported the notion that cell-to-cell contact was not the sole mechanism involved in the co-culture system as growth factor and cytokine secretions were increased in the co-culture group.

Endometrial co-culture: proposed mechanism

Various growth factors and cytokines have been associated in embryo development and implantation, among them LIF, interleukin 1, 6 and 11 (IL1, IL6, IL11), insulin-like growth factor (IGF), granulocyte-macrophage colony stimulating factor (GM-CSF), transforming growth factor α (TGFα). These factors may be secreted by endometrial cells used during co-culture to improve embryo development.

LIF was shown to promote embryo development and to increase implantation. The critical importance of LIF was first shown in mice: LIF concentrations were found to increase in mouse plasma on the implantation day and to sustain the pregnancy, as LIF null mice were infertile because of an implantation failure (Fouladi-Nashta et al., 2005). Interestingly, LIF-null embryos could implant after transfer to wild-type females demonstrating clearly that the defect resided on the maternal side (Bhatt et al., 1991). The expression of LIF mRNA and protein, predominant during the mid and late-luteal phase, is localized to the glandular and luminal epithelium in women (Sharkey et al., 1995) and is up-regulated

during the window of implantation (Bhatt et al., 1991). Interestingly, embryos from mice lacking a particular growth factor are capable of completing preimplantation development, with one exception being LIF, showing the fundamental importance of LIF in embryo development (Hardy and Spanos, 2002). Moreover, LIF was shown to significantly enhance the quality and number of human blastocysts formed in serum-free medium, promoting day 5 embryo transfer in IVF (Dunglison et al, 1996).

Interleukin1 (IL1) system has also been involved in implantation, since IL1 and its receptor are detected in the endometrium during the implantation process (Kauma et al., 1990). Furthermore, the blockage of the IL1 receptor in mice prevents the attachment of the blastocyst to the uterus (Simon et al., 1994). IL1 was also shown to induce LIF expression in human endometrial stromal cells *in vitro* (Arici et al., 1995). Therefore, beside the direct role of each cytokine on the implantation process, the interaction of each cytokine with each other seems to be also of crucial importance. Human embryos can secrete IL1β in the presence of endometrial epithelial cells, but not stromal cells, in the early stages of embryo development. IL1 induces a regular increase in cell number (De los Santos et al., 1996; Spandorfer et al., 2000). There is also direct evidence that the endometrium can respond to the human blastocyst: *in vitro* studies have shown that the human blastocyst can regulate expression of the cell adhesion molecule β3 integrin via production of IL-1β (Simon et al., 1998 and 2000).

Secretome profiling of co-culture with endometrial epithelial cells and sequential conditioned media was established using protein array membranes. Interestingly, interleukin6 (IL6) and placenta growth factor (PIGF) were the two major proteins that differ in their expression favoring the implanted embryo co-culture media (5.4 and 4.22 fold increase, respectively). A multitude of molecules was consumed (ENA78, BTC, FGF4, IL12p40, TIMP1 and uPAR). As the most dramatic change was observed for IL6, a further ELISA test was applied to confirm the previous results and to quantify the IL6 consumption. IL6 was abundant in the culture media of the embryos that did not implant, leading the authors to suggest that a healthy embryo consumes IL6 and that consequently, IL6 concentrations may be used as “an embryonic viability marker” (Dominguez et al., 2010).
Insulin-like growth factor (IGF) is secreted by endometrial stromal cells and has a role in early stages of embryological development. Human embryos express only the receptors for IGF1 but not the ligands, IGF1 is therefore a good example of the paracrine action of the reproductive tract on the developing embryo. It improves the proportion of embryos developing to the blastocyst stage by 25%, with these blastocysts having an increased number of cells constituting the inner cell mass (ICM) and an inhibition of cell death (50% significant reduction in apoptosis) (Spanos et al. 2000). Expression of IGF1, IGF2, IGF1-R, IGF2-R, and insulin-receptor were investigated by RT-PCR (reverse transcriptase polymerase chain reaction) in donated human embryos (Liu et al., 1999). Although the expression of each was increased in the co-culture group, IGF1 and IGF1-R were significantly more expressed, suggesting a key role in early embryo development. The co-culture group was defined as having an improved embryo quality, a better morphology, with more cells in the ICM.

Furthermore, Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) promotes blastocyst formation and increases ICM cell number (by 35% in Sjöblom et al., 1999). It also augments blastocyst hatching and implantation rate (Spandorfer et al., 1998; Robertson et al., 2001). TGFα (Transforming Growth Factor) has been also involved in the development of the embryo as in culture, TGFα allows for blastocoele expansion and its absence generates a high rate of apoptosis. (Osterlund et al., 2001).

Another mechanism proposed for endometrial co-culture, although indirect, was the induction of decidualization of the endometrium by scratching, inducing secretion of cytokines and growth factors involved in wound healing. The possibility that local injury of the endometrium

increases the incidence of implantation was explored (Barash et al., 2003). Transfer of a similar number of embryos increases the chance for a take-home baby by twofold in the endometrial biopsy group. Implantation and clinical pregnancy rates were 27.7% vs. 14.2% (p=0.00011), and 66.7% vs. 30.3% (p=0.00009), in the biopsy and control group, respectively. An abstract reported then a study enrolling 300 patients, who underwent a biopsy in the cycle before IVF, while the remaining 365 underwent their biopsy at least more than two cycles prior to their IVF cycle (Spandorfer et al., 2005). No significant difference was found in clinical pregnancy (43.9% vs. 40.7%) or implantation rates (17.0% vs. 16.1%), respectively.

The preimplantation embryo is free-floating and is moved continuously in the uterine fluid during the first week. Blastocyst implantation can therefore only be achieved through an effective maternal-embryonic dialogue. A bidirectional secretion of soluble mediators like cytokines and growth factors regulate the adhesion of day 6-7 blastocyst to the endometrial epithelium. During implantation, the uterine tissue, controlled by ovarian steroids, releases a multitude of cytokines in a time-specific manner. Uterine-derived signals maintain a harmonious blastocyst development and activate embryonic signaling (Armant, 2005). Endometrial co-culture gives the possibility to mimic this *in vivo* environment and hence, to initiate the maternal-embryonic dialogue before the embryo is replaced in the uterus. Indeed, clinical data obtained with autologous endometrial epithelial cells have proven to improve the pre-embryo quality.

Clinical results

In the beginning of the 90's, as simple culture media used were not able to support embryo growth through the blastocyst stage, the consensus was to transfer the preimplantation embryos on day 2 or 3 when they were at the 2-to-8 cell stage. However, the main handicap was to transfer cleavage stage embryos in the uterus in a period when they should be physiologically in the fallopian tube. The idea of growing the embryo in a nearly physiologic milieu, optimizing the *in vitro* environment to mimic *in vivo* conditions was therefore rapidly accepted and tested by many research groups. An autologous co-culture system, including stroma and glandular epithelial cells, was then introduced (Nieto et al., 1996; Barmat et al., 1998). Endometrial biopsy was performed in the luteal phase, in a cycle before the patients's IVF procedure. Obtained and purified stromal and glandular cells were passaged and cryopreserved for further use as a feeder layer. Zygotes obtained were allocated to grow randomly either on autologous endometrial co-culture or in conventional medium. The number of blastomeres of each pre-embryo was found to be greater in the co-culture group (7.4±1.3 vs 6.7±1.9; p=0.032) and fragmentation rates were found to be reduced (21%±13 vs. 24%±11; p=0.045). The conventional medium used was human tubal fluid supplemented with 15% maternal serum, and as discussed by the authors themselves, it was not supplemented with glutamine or EDTA, and the Na^+ / K^+ ratio was not well defined yet, nor were the glucose requirements of the developing pre-embryo (Barmat et al., 1998). Furthermore, they stressed that the conventional medium was devoid of growth factors.

Co-culture using human autologous endometrial cells with a blastocyst stage transfer has been also evaluated in patients with implantation failure, defined as at least three previously failed cycles with three or four good quality embryos transferred in each IVF cycle (Simon et al., 1999). The study was designed to compare patients with implantation failure, with their own oocytes or oocyte donation, compared to a routine day 2 transfer. Interestingly, in the ovum donation patients the implantation rate was significantly improved (32.7% vs. 4.5%), whereas the patients undergoing IVF with their own oocytes and blastocyst transfer have a mean implantation rate of 11.9% vs. 10.7% in day 2 transfer without co-culture. The authors suggested that the ovulation induction drugs used in IVF produced endometrial alterations leading to suboptimal receptivity. They concluded that embryonic quality makes the difference when the endometrial receptivity is optimal, setting the endometrial receptivity as a limiting factor in IVF success. Moreover, an

improvement was also reported in pre-embryo quality in the autologous endometrial co-culture group compared to the non-co-cultured group in another study enrolling 26 couples compared to their previous IVF attempt. The mean number of blastomeres was increased (6.1±1.8 vs. 4.9±1.3 cells; p=0,01) and cytoplasmic fragments were decreased (14%±10 vs. 22%±15; p=0,003). The delivery rate obtained was 73.1%. (Spandorfer et al., 2002).

An important issue when discussing the success of co-culture with autologous human endometrial cells in improving pregnancies is the biopsy date, since the molecules secreted in the co-culture by the endometrial cells may fluctuate according to the day of the cycle the biopsy is performed. Late luteal biopsies (>5 days after LH surge) have been shown to improve the number of blastomeres, the fragmentation rates and most importantly the pregnancy rate compared to early luteal biopsies and to conventional media in a total of 208 patients with multiple IVF failures. This has again demonstrated that improvements observed in co-cultured embryos were not solely due to the establishment of cell-to-cell contacts but prominently to autocrine and paracrine secretions supplied by the co-culture (Spandorfer et al., 2002).

As the use of non-human cell lines has raised concerns about the possible presence of not yet identified viruses and eventually prions that can be passed on to humans, the U.S. Food and Drug Administration has recommended in April 2002 not to use non-human cell lines for co-culture in IVF. Therefore, the co-culture trend in human IVF was interrupted and the number of studies published afterwards has significantly diminished (Kattal et al., 2008). In addition, reproductive research has meanwhile significantly progressed in not only stimulation protocols, collection, selection and micromanipulation techniques of gametes, but also in culture systems for the human embryo.

A meta-analysis published in 2008 describes in detail the inconsistencies existing between co-culture studies (Kattal et al., 2008). As improvement in pre-embryo grade, increase in mean number of blastomeres and decrease in fragmentation rates obtained in co-cultured embryos have been either obtained with human or non-human various cell lines, a cell type specificity may be ruled out. This report questions the quality of co-culture study designs and underlines the discrepancies existing in the co-culture literature due to a small sample size and an absence of a prior power analysis. The data of prospective randomized trials included in the meta-analysis was pooled: the co-culture group was favoured for implantation (3% increase with a 95% CI 0.3-5.7), clinical pregnancy (8.1% increase; with a 95% CI 2.7-13.4) and ongoing pregnancy rates (8.7%; with a 95% CI; with a 95% CI 2.8-14.6). Furthermore, the number of blastomeres per embryo was increased in the co-culture group. The heterogeneity of the cell lines and of the culture media is the major variables in co-culture studies (Kattal et al., 2008), hence randomized controlled trials are needed to compare embryo culture media with co-culture systems. A study has been subsequently published (not included in the meta-analysis) comparing development of embryos from the PGD program in co-culture with human endometrial epithelial cells or in sequential media and a blastocyst stage transfer, in both own and oocyte donation patients (Dominguez et al., 2010). The blastocyst rates were significantly higher in the co-culture group when compared to the sequential media, for the embryos of the PGD program (56% vs. 45.9%) and a similar increase was found for the ovum donation group (70.5% vs. 56.4%). Pregnancy (50.7% vs. 45.2%) and implantation rates (39% vs. 32.2%) were also found to be superior in the co-culture group.

A non-contact co-culture setting, referred to as “Transwell system”, was developed for IVF purposes (Desai et al., 2008). A permanent human endometrial cell line was established (Desai et al., 1994; Desai and Goldfarb, 1996), and proven to be comparable to both Vero cells and human oviductal cells. Human endometrial cells were seeded into the outer well of the Transwell dish, whereas the embryos were

placed onto the insert, preventing direct contact between endometrial cells and the embryos. The advantages of this method are numerous: (i) the Transwell system circumvents the anticipated biopsy, which can be stressful for the patient (ii) Utilizing a permanent human endometrial cell line may allow for an efficient standardization of the co-culture technique across patients, precluding patient-to-patient variation discussed earlier (iii) Embryos can benefit from the embryotrophic factors secreted by the co-cultured cells, and dispose of free radicals and other toxic metabolites, and still remain totally isolated through a non-toxic membrane. Using the Transwell co-culture system in 316 poor prognosis IVF patients, authors reported a clinical pregnancy rate of 53% in women less than 39 years of age and of 33% in women between 39-42 years. The enormous efforts invested in improvements of the culture media, unfortunately, have not been accompanied by studies determining the appropriate tool to hold embryos during *in vitro* development. Although it is well known that embryos are surrounded *in vivo* by the microvilli of the oviductal epithelium, studies investigating the advantages of a three-dimensional embryo culture system are still sparse and have not obtained wide acceptance. Of note, Roh et al. (2008) obtained higher blastocyst rates in murine embryos in a microtube culture system, without oil overlay, compared to the traditional drop culture system.

In conclusion, the co-culture system is designed to culture the fertilized gamete cells with the special somatic cells amplified by culturing, as a single layer in the same media until the time of embryo transfer, to improve embryo development. Various mechanisms to explain the advancement in embryo development and quality in co-culture systems have been proposed: cytokine, growth factors, trace elements elaboration not present in defined media; cell-to-cell interactions; removal of potentially harmful substances such as heavy metals, ammonium, free radicals. However, the nature of the cross-talk between embryos and feeder cells still remains largely unknown.

Experience of the Istanbul Memorial Hospital Endometrial Co-culture Program

A subgroup of patients with good quality embryos and prepared receptive endometria are well-known to fail repeatedly ART attempts. In those repeated implantation failure (RIF) cases, with more than two unsuccessful cycles, to initiate the maternal-fetal dialogue before transfer and to increase the probability of implantation, new strategies in culture systems have been developed, and co-culture models have been widely used. We propose, in Istanbul Memorial Hospital, Assisted Reproductive Technologies and Reproductive Genetics Center, endometrial co-culture to RIF patients and for the isolation of endometrial cells we apply a simplified version of the protocol from Barmat et al. published in 1998. First passaged-cryopreserved stroma and glandular epithelial cells are co-cultured with day 1 pre-embryos.

Isolation of glandular and stromal cells from endometrial biopsy tissue

Luteal phase endometrial biopsy is performed with use of a Pipelle (Endocell, France) in a cycle before ART. The tissue is washed to remove excess red blood cells and mucus, minced into small pieces (1-2mm^3) and incubated at 37°C for 30 min with 0,25mg/ml collagenase type II in co-culture medium containing RPMI 1640 medium (with L-Glutamin), 1% PSA (penicillin, streptomycin, amphotericin) and 10,1% denatured maternal serum, by gently inverting the tube every 5min. The digested tissue pieces are allowed to settle by differential sedimentation at unit gravity. After sedimentation, the pellet containing gland cells and the supernatant containing a mixture of single stromal cells and small intact glands are transferred into separate 15ml polyethylene test tubes. Stromal cells are then centrifuged for 5 min at 1500g and the supernatant is discarded. The pellet containing gland cells is supplemented with 5ml collagenase solution, prepared as described above, also centrifuged for 5 min at 1500g, and the supernatant is transferred to the stromal fraction. Each fraction is resuspended in 4ml co-culture medium (RPMI-10% patient serum) and plated separately into 50ml tissue culture flasks.

Cells are frozen when they reach confluence. They are trypsinized, suspended in a DMSO-containing freezing solution with a 1:2 ratio, and the cryovial is plunged in liquid nitrogen (modified from Barmat et al., 1998). Cells are thawed 3 days before the oocyte pick-up day. Stromal and gland cells are then mixed in a 1:1 ratio and plated together to a Nunc 4-well dish (3.10^5cells/well) (Fig. 1). When pronuclei become visible on day1, preembryos are placed on the gland-stromal cells and incubated at 37°C in a GPS dish until blastocyst stage. (Fig. 2).

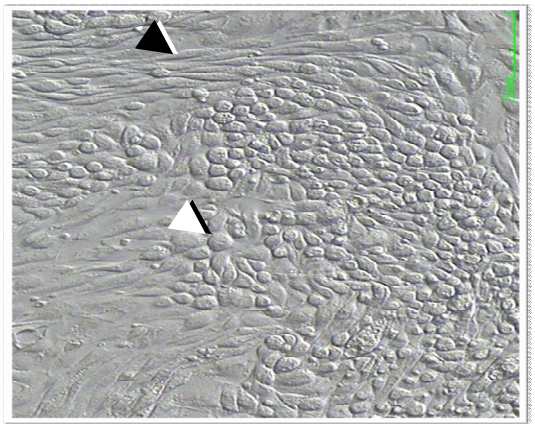

Fig. 1. Invert microscope view of stromal (black arrow) and glandular (white arrow) cells in culture. Endometrial stromal cells are spindle-shaped with many extensions, whereas glandular cells are round and have no extensions (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

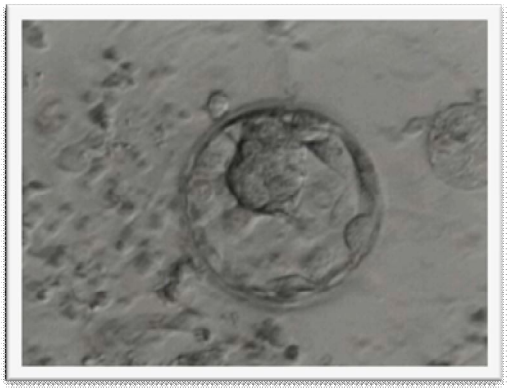

Fig 2. Blastocyst co-cultured with endometrial cells forming a monolayer on the bottom of the culture flask (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

A small aliquot of the gland/stromal cells fraction was diluted 1:1 with trypan blue stain (4%), and cell yield and viability were determined quantitatively on a hemocytometer (Fig. 3).

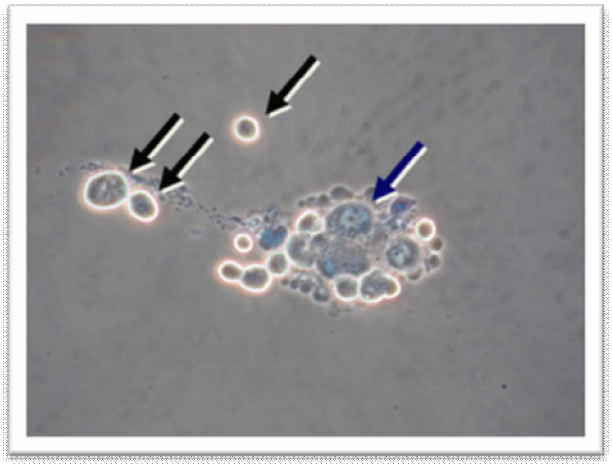

Figure 3. Trypan blue staining (4%) (Sigma T-8154). Cells stained with blue are dead (blue arrow) and unstained cells are vital (black arrows) (x400) (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

Immunofluorescence characterization of stromal and glandular epithelial cells was performed with vimentin and cytokeratin staining. Vimentin is one of the major intermediate filaments found in cells and is a specific marker for mesenchymal cells. Cytokeratin filaments are located within the cytoplasm of cells that are predominantly found of epithelial origin. Glandular and stromal cells were seeded in a 1:1 proportion as a monolayered tissue culture (Figure 4a, b).

As presented in the 3rd World Congress of the Association of Reproductive Medicine (W.A.R.M.) in 2006, we have conducted a clinical study on whether autologous endometrial co-culture is beneficial for patients with multiple assisted reproductive treatment failures with defined embryo quality. Embryos from RIF cases were randomly allocated to grow either on autologous endometrial cells (n=389) or on

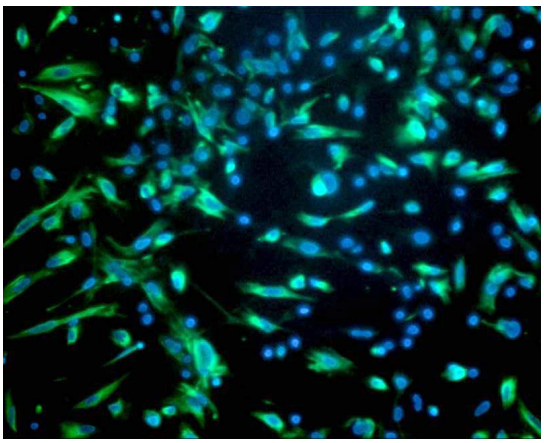

Figure 4a. Stromal cells were stained with vimentin antibody (green labeling). DAPI stains all nuclei in blue, therefore cells with blue staining only are glandular cells (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

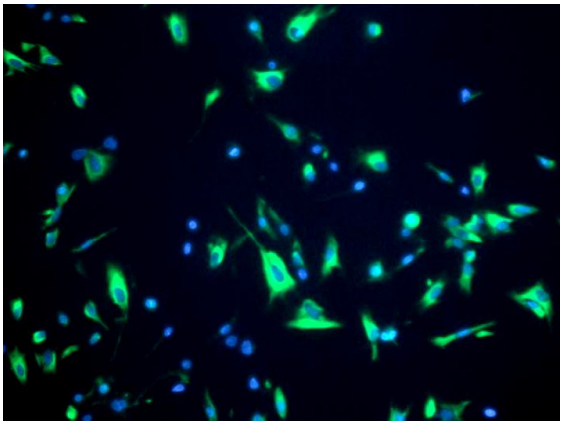

Figure 4b. Glandular cells were stained with cytokeratin antibody (green labeling). DAPI stains all nuclei in blue, therefore cells with blue staining only are stromal cells (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

autologous endometrial cells (n=389) or on Life Global culture medium (n=369). Day 3 embryo development was assessed in each study group, and our unpublished results are as follows: cleavage arrest rate was found to be higher in the control group compared to the co-culture group (21.45% vs. 7.35% respectively; $p<0.01$). Furthermore, the rate of embryos with more than six blastomeres was significantly increased in the co-culture group (68.9% vs. 44.7%), with 10.4% less fragmentation (16.7% vs. 27.1%; $p<0.05$). Endometrial co-culture was also found to increase pregnancy rate (PR) in RIF cases (n=389) compared to the control group (n=369), with transfers being conducted mostly on day3 for both groups (43.7% for the co-culture and 51.1% for the control group). Interestingly, PR was significantly increased in patients with two to four previous conventional ART attempts undergoing co-culture, when compared to the control group. For two previous attempts PR was 44.1% in the co-culture group (n=90) and 36.3% in the control group (n=84). For three attempts PR was 41.8% (n=83) and 24.3% (n=86), respectively, for four attempts 37.5% (n=54) and 24.7% (n=45), respectively ($p<0.05$). For patients with five or more previous attempts, co-culture does not bring any benefit, as the cause of ART failure might not be related to embryo culture directly (n=80 and n=74, in the co-culture and control group, respectively).

In conclusion, promising results have been obtained regarding the co-culture of embryos on feeder layers of human endometrial cells. Decreased fragmentation, as well as increased blastomere numbers were reported, and as a result, co-culture of human embryos on autologous endometrial epithelial cells has been included in the clinical programme in several centres worldwide, especially for patients with multiple assisted reproductive treatment failures. However, co-culture technique remains labor-intensive and its effectiveness vary from patient-to-patient and with the considerable improvement of culture media, compensating for the special needs embryos, further randomized controlled studies are warranted to provide evidence on the efficacy of endometrial co-cultures.

On the other hand, the concept of cells helping cells has proved to be successful not only in human embryo development but also to derive human embryonic stem cell lines, and better results are obtained when feeder cells are used to support human embryonic stem cells (Figure 5) (Candan and Kahraman, 2010).

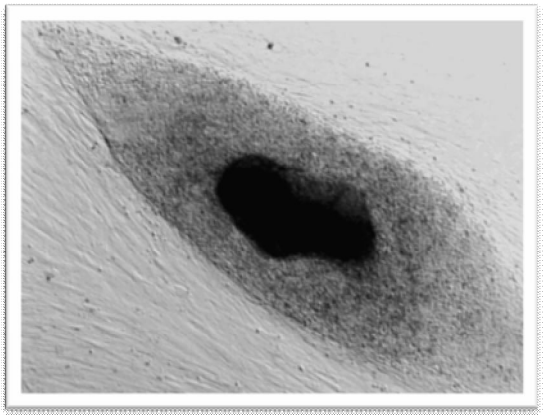

Figure 5. Embryonic stem cells growing on a monolayer of endometrial stromal cells (Istanbul Memorial Hospital, R&D Laboratory, Turkey).

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