Article

Is there still a place for co-cultures in the era of sequential media?

After his residency training in Obstetrics and Gynecology in the University of Hacettepe in Ankara, Dr Urman completed a 3-year fellowship programme in Reproductive Endocrinology and Infertility in Vancouver, Canada. He returned to Hacettepe University in 1991 and participated in the foundation of one of the first IVF clinics in Turkey. He worked as an Associate Professor until 1996 in the same institution. Dr Urman resigned from the university in 1996 and founded the Assisted Reproduction Unit of the American Hospital of Istanbul, one of the biggest IVF centres in the country. His major areas of interest are clinical assisted reproduction, laparoscopic and hysteroscopic surgery. He has published extensively in these fields, having over 70 articles published in renowned international journals.

Dr Bulent Urman

Basak Balaban obtained her BSc in 1993 from the University of Ankara, Turkey. Her subsequent clinical embryology and IVF training took place in the Ankara Sevgi Hospital and the Schoysman Infertility Management Foundation in the team who had pioneered the first intracytoplasmic sperm injection and testicular sperm extraction in Turkey. She founded the embryology laboratory in the VKV American Hospital of Istanbul in 1996. She is the Director and principal embryologist in this laboratory that performs over 1000 IVF/ICSI and PGD cycles per year. Her laboratory research interests include blastocyst culture, in-vitro spermiogenesis and cryopreservation. She has in excess of 30 publication to her name.

Basak Balaban, BSc

Bulent Urman[1], Basak Balaban
Assisted Reproduction Unit, American Hospital of Istanbul, Turkey
[1]Correspondence: Guzelbahce sok No 20, Nisantasi, Istanbul, Turkey; e-mail: burman@superonline.com

Abstract

Co-cultures have been advocated in assisted reproduction owing to the inadequacy of simple media to support embryo development beyond the cleavage stage. Different human and non-human cells and cell lines have been used for co-cultures. High rates of blastocyst formation have been reported with the use of co-cultures, and they have been proposed as a salvage treatment option in couples with repeated implantation failures. Since the advent of complex sequential media, which yield very high blastocyst formation and blastocyst implantation rates, the need for co-cultures has been questioned. Upon review of the literature, it is evident that well-designed randomized studies that compare co-cultures with simple or sequential media do not exist. Progression to the blastocyst stage for cleavage stage embryos appears to be similar, if not better, for embryos that are cultured in modern sequential media, rendering the use of co-cultures obsolete. Furthermore, there is no consensus regarding the necessity of sequential media, as similar results have been obtained with a single medium formulation that supports all stages of the preimplantation period. Whether co-cultures are beneficial in patients with repeated implantation failures, however, should be investigated in randomized trials. Co-cultures still serve as powerful tools for understanding embryo metabolism. Furthermore, co-cultures may be instrumental in studying expression of implantation-related genes and embryo–endometrium interaction.

Keywords: *blastocysts, co-cultures, culture media, human embryos, implantation*

Introduction

Co-cultures have been advocated in assisted reproduction owing to the inadequacy of simple media for supporting embryo development beyond the cleavage stage. Multiple cell lines have been used in a co-culture system, ranging from human reproductive tissues to non-human cells or cell lines (Ménézo *et al*., 1990, 1995; Bongso *et al*., 1991, 1994a,b; Wiemer *et al*., 1991; Plachot *et al*., 1993a, 1994; Saito *et al*., 1994; Schillaci *et al*., 1994; Freeman *et al*., 1995; Jayot *et al*.,

1995; Quinn and Margalit, 1996; Yeung *et al*., 1996). Some of these are tubal or endometrial epithelial cells (from humans or animals), autologous cumulus or granulosa cells, or an established cell line such as monkey kidney (Vero) cells. Suggested benefits of co-cultures include secretion of trophic factors such as nutrients, substrates, growth factors and cytokines, and removal of potentially toxic substances from the culture medium by co-cultured cells. Composition of the culture media appears to be influenced favourably by the somatic cells. Edwards and co-workers showed a reduction in glucose and an increase in lactate and pyruvate concentrations of the culture media in the presence of bovine oviductal epithelial cells (Edwards *et al*., 1997). However, the use of inappropriate volumes for co-culture can acidify the medium through the glycolytic activity of the somatic cells. Before the late 1990s, most of the embryos generated in the IVF laboratory were placed into the uterus at the cleavage stage. The reason for this was the inability of older culture systems to support the development of viable blastocysts at acceptable rates. When embryos are selected for transfer at the cleavage stage, the embryonic genome has only just begun to be transcribed, and therefore it is not possible to identify from within a given cohort the embryos with the highest developmental potential. However, when blastocyst culture is undertaken, those embryos that are destined to stop their development can be identified.

In humans, blastocyst culture has been reported to substantially increase the implantation rate per embryo transferred (Gardner *et al*., 1998; Huisman *et al*., 2000; Milki *et al*., 2000). Highest implantation rates reported for cleavage stage embryo transfers are lower than those reported for blastocyst transfers (Van Royen *et al*., 1999; Gardner *et al*., 2000). Advantages of blastocyst transfer include better synchronization of the embryo with the endometrium, better assessment resulting in selection of the most implantation competent embryo, selection against certain chromosomal defects, and decreased uterine contractility and cervical mucus at the time of transfer (Olivenness *et al*., 1994).

Optimal conditions for the early stage embryo do not support more advanced embryo development. Moreover, when blastocysts do arise from cleavage stage embryos under these suboptimal conditions, implantation rates are severely reduced, suggesting diminished viability. In simple media supplemented with pyruvate and serum, 40% of cleavage stage embryos were able to reach the blastocyst stage (Bolton *et al*., 1991). However, only 7% of these blastocysts implanted into the uterus. The relatively low rate of blastocyst formation and blastocyst implantation derived from embryos cultured in simple media led to popularity of co-cultures during the early and mid-1990s. In mostly uncontrolled and non-randomized studies (Plachot *et al*., 1993a; Bongso *et al*., 1994a,b; Schiallaci *et al*., 1994; Freeman *et al*., 1995), embryos co-cultured with various feeder cells led to the production of implantation-competent blastocysts.

Co-cultures and blastocyst formation

Vero cells were reported to improve the pregnancy rate in patients with previous implantation failures compared with patients undergoing IVF–embryo transfer for the first time (Schillaci *et al*., 1994). However, a randomized study published concomitantly showed no difference in the outcome of IVF when embryos were co-cultured with Vero cells compared with simple media (Sakkas *et al*., 1994). Only patients undergoing IVF for the first time were randomized. The authors elected to allocate all patients who had repeated implantation failures to co-culture. These findings were confirmed by another study that compared co-culture with simple media (Van Blerkom, 1993). The authors prospectively randomized excess embryos from 100 patients to be cultured from the 2-cell stage to the blastocyst stage in the presence or absence of Vero cells. Several embryonic parameters, such as fragmentation, developmental arrest, multinucleation and blastocyst formation, were observed in a time period of 7 days. With respect to these developmental parameters, the authors were unable to show statistically significant improvement in early human embryogenesis in the co-culture system.

In a subsequent study, a total of 70 supernumerary human embryos from 15 patients were divided randomly between two culture conditions: (i) co-culture with Vero cells; and (ii) culture in the medium routinely used in the centre (Turner and Lenton, 1996). Regarding blastocyst formation rates, embryos cultured on Vero cells scored better than their counterparts cultured in normal media.

Implantation of co-cultured embryos

Implantation of co-cultured cleavage stage embryos on cumulus cells, isolated granulose cells, oviductal cells, endometrial cells, and even sequentially in oviductal and endometrial cells have been reported. Most studies show a beneficial effect of co-cultures in terms of embryo quality and progression to the morula and blastocyst stages (Ménézo *et al*., 1990; Plachot *et al*., 1993a; Bongso *et al*., 1994a; Tucker *et al*., 1994; Freeman *et al*., 1995; Jayot *et al*., 1995; Quinn and Margalit, 1996; Yeung *et al*., 1996; Barmat *et al*., 1999; Carrell *et al*., 1999). However, results are discrepant regarding embryo viability. Plachot and co-workers showed that granulose cell co-culture increased the progression of cleavage stage embryos to the blastocyst stage in couples with repeated implantation failures (Plachot *et al*., 1993b). However, these embryos did not implant more efficiently than their non-co-cultured counterparts. In a subsequent study, Quinn and Margalit (1996) cultured human embryos with their cumulus cells in insemination drops, which differed only in the presence or absence of homologous cumulus cells. A significantly greater proportion of expanded blastocysts developed in the co-culture group. Other investigators also showed beneficial morphological effects of autologous cumulus cells when used to co-culture supernumerary embryos obtained through IVF (Fabbri *et al*., 2000).

Tucker and colleagues compared co-cultures with assisted hatching and day 3 embryo transfer in women >38 years of age or who had failed to conceive with multiple IVF attempts (Tucker *et al*., 1994). Significantly higher implantation and pregnancy rates were obtained in the assisted hatching and co-culture group, although ongoing pregnancy rates were similar. Wiemer and co-workers applied co-culture with assisted hatching in patients with repeated implantation failures and observed significantly increased blastomere numbers and decreased fragmentation in co-cultured embryos (Wiemer *et al*., 1996). Furthermore, implantation and pregnancy rates (17 and 38%) were impressive in this group of poor prognosis patients. Freeman *et al*. failed to show an increase in blastomere number