EGF and IGF-I as predictors of ICSI outcome in human preimplantation embryo cultures

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Summary

Purpose of investigation: Detection of EGF and IGF-I in human embryo cultures and their effect on ICSI outcome.

Methods: Collection of culture medium from embryos of 50 women under ICSI program. EGF and IGF-I were measured via enzyme immunoassay.

Results: ICSI outcome was independent of age, infertility years, FSH, LH, prolactine and E2. EGF detection was higher in 48 - (32%), than in 72-hour embryos (14%) ($p < 0.001$). EGF negative embryos are likely to be arrested at the morula stage ($p < 0.001$) and are associated with poor pregnancy rates ($p < 0.05$). IGF-I was undetected in 48-hour embryos.

Conclusions: For the first time human embryos were surveyed from fertilization until embryo transfer, regarding EGF and IGF-I production. IGF-I is not a predictor of ICSI outcome. EGF is present in one-third of human embryo cultures at 48 hours, but this ratio wanes at the morula stage. EGF negative embryos are associated with lower pregnancy rates.

Key words: Assisted reproduction; ICSI; Human preimplantation embryos; EGF; IGF-I; Predictors.

Introduction

The earliest cell cycles of the embryo are characterized by successive cleavages that take place automatically, under the influence of information derived from the oocyte cytoplasm [1]. Activation of zygotic genetic material takes place later (maternal to zygotic transition) [1, 2]. These successive cell cleavages that follow the fertilization of the oocyte are not caused by specific growth factors, as the embryo seems to auto-stimulate its divisions [1]. However, preimplantation embryos have the ability to respond to growth factors that are artificially added to the culture.

In an attempt to study the factors that control early embryo divisions and to study their prognostic significance in fertilization and cleavage rate, quality of embryos as well as the resulting pregnancy rates, we evaluated the presence of epidermal growth factor (EGF) and insulin growth factor I (IGF-I) in human early preimplantation embryo culture medium.

Materials and Methods

A total of 50 consecutive patients, aged 25 to 43 years old, with a history of one to six previous attempts of intracytoplasmic sperm injection (ICSI) protocols and with a normal hormonal profile, were included in the present study. All women provided informed consent to be included in the study, which took place under the approval of the ethics committee of the University of Athens.

The stimulation protocol was commenced with rFSH at a fixed dose of 200 IU daily. Embryos were graded according to their morphologic appearance, on the day of transfer. One to four embryos were transferred on day 3 after retrieval. Serum hCG was measured 14 days after oocyte retrieval. Embryos were cultured in groups of four to six embryos in petri dishes for 72 h after the ICSI procedure, before embryo transfer. Culture medium from 388 human embryos were obtained at 48 hours and 72 hours after ICSI.

EGF and IGF-I levels were measured using a quantitative sandwich enzyme immunoassay technique (Quantikine, R&D systems). Plates were read and results were obtained using a microprocessor-controlled photometer system (Stat Fax 2100, Awareness Technology Inc.). Minimum detectable doses were 0.007 ng/ml and 0.7 pg/ml for IGF-I and EGF detection kits, respectively. In each assessment, culture medium was used as negative control.

Statistical analysis included the t-test for comparison of means and x^2 for proportions; values of $p < 0.05$ were considered statistically significant. Patients were allocated to groups according either to the in vitro fertization (IVF) outcome (pregnancy and non-pregnancy), or to the production of EGF (EGF positive and EGF negative cultures), or IGF-I (IGF-I positive and IGF-I negative cultures).

Results

Women with successful and unsuccessful pregnancy outcomes did not differ significantly with respect to age and duration of infertility (Table 1). Basal levels of FSH, LH and prolactine on the third day of the cycle were also indifferent for the two groups (Table 1). Serum estradiol on the day of hCG administration was 2781 pg/ml (± 1760) and 2142 pg/ml (± 722) for the pregnancy and non-pregnancy groups, respectively, showing no statistically significant difference (Table 1). No statistical difference was evidenced regarding the number of follicles and oocytes retrieved per patient as well as the percentage of mature oocytes and the fertilization rate.

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Table 1. — *Clinical and hormonal features of the patients comparing women resulting in pregnancy and women not resulting in pregnancy.*

	Non Pregnancy group (32)	Pregnancy group (18)	Significance
Age	34.2 ± 4.6	32.4 ± 3.9	n.s.
Years of infertility	6.3 ± 5.1	6.0 ± 3.2	n.s.
FSH (mIU/ml)	6.06 ± 2.08	5.85 ± 2.11	n.s.
LH (mIU/ml)	5.20 ± 1.77	7.27 ± 5.49	n.s.
Total dose (IU)	2406.6 ± 1096.4	2075.0 ± 564.4	n.s.
Estrogen[a] (pg/ml)	2781.2 ±1760.0	2142.0±722.4	n.s.

[a]: On the day of hCG administration; n.s.: not significant.

Samples of culture medium that were used as negative controls had undetectable EGF and IGF-I. EGF was detected in cultures from 16 out of 50 women (32%) at 48 hours of culture and in seven out of 50 women (14%) at 72 hours of culture ($p < 0.001$). Mean EGF levels of EGF positive embryo cultures were 1.4 pg/ml (± 0.2). Cross tabulation, using chi square criterion showed that women with undetectable EGF at 48 hours are more likely to present arrested embryos at 72 hours ($p < 0.001$) and are more commonly associated with lower pregnancy rates ($p < 0.05$) (Table 2).

Table 2. — *Clinical data comparing patients who were found to have EGF producing embryos and EGF negative embryos.*

	EGF negative at 48 hours	EGF positive at 48 hours	Significance
Age	33.2 ± 4.2	34.1 ± 4.9	n.s.
Years of infertility	6.1 ± 3.2	6.5 ± 6.4	n.s.
FSH (mIU/ml)	5.9 ± 2.2	6.2 ± 1.8	n.s.
LH (mIU/ml)	5.9 ± 3.6	5.6 ± 3.3	n.s.
Total dose (IU)	2116.6 ± 660.5	2618.7 ± 1320.6	n.s.
Estrogen [a] (pg/ml)	2372.6 ± 909.2	2958.0 ± 2251.0	n.s.
Pregnancy rate (%)	21	50	$p < 0.05$

[a]: On the day of hCG administration; n.s.: not significant.

IGF-I was undetected in culture medium from embryos at 48 hours, whereas it was detected in cultures of embryos from nine women (19%). In the latter, mean IGF-I levels were 0.3 ng/ml (± 0.4). Embryos positive for IGF-I were equally distributed in successful and unsuccessful outcomes.

Discussion

The observation that embryos possess the property to respond to growth factors that are artificially added to the culture media is not new [3, 4]. Conclusions that derive directly from this observation are that early embryos express receptors that mediate such actions. The question that arises is whether embryos actually produce the same growth factors in order to act in an autocrine manner, as a means of self-control of their growth. This is the first study in which human embryos were surveyed from the fertilization point for 96 hours until embryo transfer, regarding EGF and IGF-I production.

Actions of EGF and IGF-I on embryos have been studied extensively. EGF possesses the ability to stimulate hatching and outgrowth, when added to mouse embryo culture medium [5, 6]. Both EGF and IGF-I independently enhance bovine pre-implantation development. In the present study, levels of EGF and IGF-I were measured in the culture media of early human embryos in an effort to evaluate their involvement in the process of early embryo development. A new finding that occurred is the detection of EGF in human embryo culture medium at the early stages of growth. A fraction of 32% of the women studied presented detectable levels of EGF in the culture medium of their embryos at 48 hours, which was lowered to 14% at 72 hours of culture. The finding that embryos, negative for EGF at 48 hours of culture, were more likely to become arrested at 72 hours of culture denotes a clear advantage of embryos producing EGF at 48 hours. Most likely, in these embryos, self produced EGF acts as a feedback to the EGF receptors and advances a more favorable development.

A series of studies have been conducted examining the expression of EGF in embryos. Wollenhaupt *et al.* have found weak transcripts for EGF in pig early embryos [7]. Similar findings had been found for the human species by the study of Chia *et al.,* who showed that transcripts for EGF and EGF receptor, were detected in 8-cell embryos and in human unfertilized oocytes [8]. These findings show that there is a degree of involvement of the EGF system in the process of embryo growth, the extent of which is yet unknown. For example, Goldman and Gonen have showed that mouse embryo growth can be interrupted by adding monoclonal antibodies against EGF in culture media [5].

The finding that IGF-I was not detectable in 48-hour embryo cultures is in accordance with the absence of the transcript from early human pre-implantation embryos that has already been published elsewhere [9, 10]. However, embryos respond to IGF-I by hatching and acceleration of their growth. These actions of embryos in response to the addition of hormones and factors of the IGF system are explained by studies that have documented the expression of IGF receptors on pre-implantation embryos [10]. These data directly engage the IGF network to early embryo development. In the present study, although IGF-I was detected in 19% of embryos at 72 hours, it had an equal distribution between embryos that proceeded to pregnancy and embryos that did not proceed to pregnancy. Thus it did not prove to be a predictor of the ICSI outcome.

The present study also showed that the expression of EGF fades during the transition from the 48-hour culture to the 72-hour culture. Similar findings have been shown by the study of Austgulen *et al.* concerning the expression of cytokines in embryo culture medium [11]. Their findings showed that there is a reduction in detected cytokines during the transition from the 24-hour period to the 48-hour period after insemination. Probably such a reduction is merely a reflection of the fading molecular stock that the embryo received from the oocyte cytoplasm during the transition period until the initiation of transcription of the zygotic genetic material (maternal to zygotic transition) [1, 2].

In conclusion, IGF-I polypeptide cannot be detected in human pre-implantation embryo cultures during 48 hours of culture, but can be detected in 19% of cultures in 72-hour embryos. This increase in the detection rate does not correlate with pregnancy rates and cannot therefore be used as a predictor of ICSI outcome. On the contrary, EGF can be detected in one-third of human pre-implantation embryo cultures during 48 hours of culture, but this fraction declines during the transition to the morula stage, probably as a result of the degradation of the transcripts from the maternal oocytes. These preliminary findings indicate that EGF negative embryos tend to arrest when cultured for a longer period and are associated with low ICSI pregnancy rates. Therefore, EGF can potentially be used as a marker for the prognosis of the ICSI procedure.

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