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Interleukin-1 β Production by Human Preimplantation Embryos¹

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INTRODUCTION

The field of growth factors and cytokines, and their effects on reproduction, is a rapidly growing new area of investigation (1). Immune cells and related cytokines have been shown to affect various reproductive events, such as ovulation and fertilization, as well as implantation and early embryonic development (2). Furthermore, different cellular components of the reproductive system and the developing embryo have been shown to secrete cytokines and growth factors (3–8).

Since the report by Zolti *et al.* (3), who demonstrated interleukin-1 (IL-1) activity in the conditioned media of oocytes and preimplantation human embryos, con-

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troveries exist regarding the IL-1 level of conditioned media and morphological score of the embryos (5), implantation capability (6), and ability to predict pregnancy during an in vitro fertilization (IVF) cycle (7). This study was conducted to address the present controversy regarding the production of IL-1 β by human preimplantation embryos and its correlation with subsequent embryonic development.

MATERIALS AND METHODS

Patients

The material for this study was obtained from women undergoing IVF–embryo transfer (ET). A total of nine women was studied. The indications for IVF were mechanical and unexplained infertility. The study required no modification of our routine human IVF protocols. Briefly, patients were pretreated with long-acting gonadotropin-releasing hormone agonist (GnRH-a; Decapeptyl, Ferring, Malmo, Sweden) in the midluteal phase using a long protocol. Fifteen days later, when adequate suppression was obtained, the patients underwent ovarian stimulation using human menopausal gonadotropin (hMG; Pergonal; Teva, Petah Tiqva, Israel). Gonadotropin dosage was adjusted individually according to serum estradiol (E₂) levels and vaginal ultrasound measurements of follicular diameter, obtained every 1 or 2 days. Human chorionic gonadotropin (hCG; Chorigon; Teva), 10,000 IU, was administered when the leading follicles reached a minimum of 18 mm in diameter, with a peak serum E₂ level of >2000 pM.

Oocyte Recovery, Fertilization, and Embryo Culture

Oocytes were aspirated by way of the transvaginal ultrasonographic route approximately 34 hr after hCG injection. After retrieval and identification, the oocytes were cultured in IVF Medium (MEDI Cult, Denmark) for 2 to 6 hr and then inseminated with 100,000 washed motile sperm per dish. Seventeen hours later the cumulus and corona cells were removed and oocytes were examined for signs of fertilization. At this stage, the medium was replaced and the fertilized oocytes [two pronuclei (2PN)] were separated, so that each embryo was kept in a different culture dish with 0.7 ml fresh culture medium. Approximately 24 hr later ($\pm$ 41 hr after recovery), embryos were examined and their cleavage stage (number of cells) was noted. In the

embryos that developed to the four-cell stage, and were intended for freezing 1 day later, culture medium was replaced and stored at -20°C in aliquots until assayed. Twenty-four hours later and before freezing, the embryo cleavage rate was reassessed (65 hr after initial oocyte aspiration).

IL-1 β Assay

The culture media of 40 embryos were tested in duplicate for IL-1 β levels, using a commercial kit of high-sensitivity IL-1 β sandwich enzyme-linked immunosorbent assay (ELISA) (quantikine HS R&D, Minneapolis, MN). All samples were assayed at one time to avoid interassay variations. The minimal sensitivity of the assay and the intraassay variability were <0.1 pg/ml and 7–10%, respectively. Blanks were included in all experiments.

Results are expressed as mean $\pm$ SD. Statistical analysis was performed by unpaired Student's *t* test and correlation analysis where appropriate; *P* < 0.05 was considered significant.

RESULTS

Conditioned embryo medium from the 2PN stage to the four-cell stage contained IL-1 β (Table I). No linear correlation was found between conditioned medium IL-1 β levels and subsequent embryonic development (cleavage rate).

DISCUSSION

The presence of various cytokines in conditioned medium of embryos during IVF has been reported

Table I. IL-1 β Concentration of the Conditioned Embryo Medium, According to the Cleavage Stage 48 hr After the 2PN Stage

Cleavage stage (No. of blastomeres) 48 hr after the 2PN stage	No. of embryos	Conditioned medium IL-1 β concentration (pg/ml) from the 2PN stage to 24 hr later ^a
4	4	1.63 $\pm$ 1.08
5	5	0.47 $\pm$ 0.35
6	9	1.9 $\pm$ 1.44
7	12	0.81 $\pm$ 0.56
8	10	0.65 $\pm$ 0.56

^a Mean $\pm$ SD.

previously (3–8). Zolti *et al.* (3) were the first to demonstrate IL-1-like activity in conditioned medium of oocyte corona–cumulus complex during the first 24 hr in culture, which decreased sharply after an additional 24 hr of incubation. They further demonstrated a resumption of IL-1 production in the two- to four-cell embryos, with a subsequent significant increase in the six- to eight-cell stage. In the present study, we confirmed the presence of IL-1 β in conditioned medium from the 2PN to the four-cell stage. This observation concurred with previous reports (3,5,7) and was further verified by immunohistochemical experiments showing intense IL-1 β staining in human oocytes and embryos (9).

We could not demonstrate any correlation between conditioned medium IL-1 β levels and subsequent embryonic development (cleavage rate). While Austgulen *et al.* (5) observed similar results, reporting a nonsignificant correlation between the culture medium IL-1 and the morphological evaluation and cleavage stage of the corresponding embryo, Baranao *et al.* (7) examined IL-1 β levels in conditioned media of human embryos and found a significantly higher IL-1 β conditioned medium in embryos that resulted in pregnancies. However, Baranao *et al.* (7) did not consider IL-1 β production by a single embryo, but rather, IL-1 β production by one to five preimplantation embryos cultured in the same capsule. Moreover, they correlated conditioned medium IL-1 β levels with pregnancy, a variable which involved factors other than embryonic. In our study we overcame the aforementioned limitations by culturing each embryo in a single well and correlating the IL-1 β level with the subsequent embryonic cleavage rate, rather than pregnancy. Since no correlation was found between the IL-1 β level and subsequent embryonic development, we assume that it would be prudent not to consider the use of the conditioned medium IL-1 β level as a potential parameter in predicting implantation. Further support for the present study was recently demonstrated by a study that measured IL-1 β levels in the supernatant of conditioned media of embryos grown in autologous endometrial coculture (10). That report found that the presence of IL-1 β was negatively associated with embryonic development and clinical pregnancy. Moreover, the authors were unable to determine whether IL-1 β itself interferes with successful outcome after embryo transfer or if it is a marker for undetected endometritis in the biopsy specimens.

In the present study we demonstrated the presence of IL-1 β in culture medium of preimplantation embryos. It seems plausible to suspect that IL-1 β pro-

duction by the preimplantation embryo may be regulated by various intrinsic and extrinsic stimuli, which, in turn, contribute to the “ontogenic inflammation” coined previously by Mizuno and Soma (11). This expression was used to describe the inflammation around the area of cell division growth and differentiation, which is necessary for maintenance of embryonic growth, differentiation, implantation, and maternal recognition of pregnancy. Further work in the cytokine subspecialty of reproductive immunology will enhance the understanding of cellular immune interaction with reproductive events, which may lead to new therapies designed to promote fertility and the prevention of reproductive failure.

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