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Physiology

Involvement of Interleukin-1 and the Interleukin-1 Receptor Antagonist in In Vitro Embryo Development Among Women Undergoing In Vitro Fertilization–Embryo Transfer

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***Purpose*:** To examine the roles of Interleukin-1 (IL-1) and IL-1 receptor antagonist (IL-1ra), in in vitro embryo development and subsequent pregnancy outcome.
***Methods*:** Maternal serum utilized to supplement embryo growth in IVF cycles was analyzed for the presence of IL-1 cytokines.
***Results*:** The maternal serum that was utilized to supplement the embryo media was found to have measurable amounts of IL-1β and IL-1ra.
***Conclusions*:** Relative antagonism of the IL-1 system was positively associated with embryo development and pregnancy outcome.

KEY WORDS: Embryo; endometrium; interleukin-1; interleukin-1 receptor antagonist; IVF.

INTRODUCTION

In-vitro fertilization is a complex process. After retrieval of oocytes and fertilization with sperm, the embryos are grown in the laboratory for a period of 2–5 days. Maternal serum is often added to media utilized to grow embryos prior to the transfer (1,2). Little work has been performed on analyzing the cytokines and growth factors in the maternal serum utilized in conjunction with the synthetic medium. In this study we evaluated the potential role of IL-1 and IL-1ra in early human embryo development.

The Interleukin-1 system is composed of a family of peptides. There are two agonists, IL-1α and IL-1β, and one antagonist, IL-1ra. IL-1 is secreted by many cells and is often found in the serum of patients. IL-1 receptors are located on the early human embryo (3–6). Thus, there exists the potential for interactions between serum IL-1 with the early embryo. Specifically, because embryos are often cultured in media supplemented with maternal serum, we investigated the effect of IL-1α, IL-1β, and IL-1ra in maternal sera in patients undergoing conventional IVF.

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MATERIALS AND METHODS

IL-1 Cytokines in Maternal Serum

We evaluated 160 patients undergoing conventional IVF. We retrieved stored maternal serum (from matched pregnant and not-pregnant patients after IVF) that had been used to supplement human tubal fluid (HTF) media for in vitro embryo growth. We analyzed for IL-1α, IL-1β, and IL-1ra and correlated the findings with embryonic development and IVF outcome. These patients were matched for age, stimulation protocol, number of previous attempts, stimulation response, and the number of embryos transferred. Sera collected for media supplementation drawn 1 day before oocyte retrieval (or 1 day post

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hCG administration), were assayed utilizing commercial ELISA kits. Embryo grade was on a scale of 1–5 (1 = best). High quality embryos were defined as grade 1 or 2.

IVF Methods. Patients were treated with standard ovulation induction protocols and underwent IVF-ET as previously described (7). In brief, all women were treated with luteal phase leuprolide acetate (Lupron; Tap pharmaceuticals, Deerfield, IL, USA), 1 mg s.c. daily until ovarian suppression was achieved. The patients had previously demonstrated normal ovarian reserve. Ovarian stimulation was then effected with a combination of gonadotrophins [human menopausal gonadotrophin (HMG), pure follicle stimulating hormone (FSH), or both] employing a step-down protocol. Human chorionic gonadotrophin (HCG) was administered (3300–10000 IU) when at least two follicles reached or exceeded 16–17 mm mean diameter as measured by transvaginal ultrasound. Oocytes were harvested by transvaginal ultrasound-guided follicular puncture 35–36 h after HCG administration.

Conventional oocyte insemination or micromanipulation was performed as indicated. Morphologically normal embryos were transferred into the uterine cavity approximately 72 h after retrieval. The number of embryos transferred was dependent on maternal age, according to our standard protocol. In general, three embryos were transferred to patients under 34 years of age, patients 34–39 years of age received four embryos, and patients over 40 underwent transfer of up to five embryos when available. Methylprednisolone (16 mg/day) and tetracycline (250 mg every 6 h) were administered for 4 days to all patients commencing on the day of oocyte retrieval. Progesterone supplementation was initiated on the third day after HCG administration (25–50 mg i.m./day) and was continued until the sonographic assessment of the pregnancy.

IL-1 ELISA. IL-1 (IL-1α, IL-1β, IL-1ra) ELISA was performed on all 160 samples utilizing commercially available kits. For IL-1α: The interassay and intraassay variation was 5.2 and 3.5%, respectively. The minimum level of detection was 0.5 pg/mL. For IL-1raβ: The interassay and intraassay variation was 5.5 and 5.5%, respectively. The minimum level of detection was 14 pg/mL. For IL-1β: The interassay and intraassay variation was 5.4 and 3.9%, respectively. The minimum level of detection was 0.5 pg/mL. Curves were generated from standards provided in the kit, and the values were determined from these curves. IL-1 (IL-1α, IL-1β, IL-1ra) was measured in each of the supernatants as well as media alone (Ham's F-10 supplemented with 15% patient serum). Prior to performing the assay, the supernatants were brought to room temperature. Cross-reactivity with other cytokines was insignificant.

Statistical Analysis. Data is presented as mean ($\pm$SD). The data was not normally distributed, and therefore, continuous data were compared utilizing nonparametric tests. Categorical data was compared utilizing chi square analysis. A p value <0.05 was considered significant.

RESULTS

IL-1 Cytokines in Maternal Serum

All the patients underwent a luteal leuprolide stimulation cycle and had normal random day 3 levels before beginning their stimulation. There were no differences in the pregnant and nonpregnant patients with respect to age, number of previous stimulations, peak estradiol levels, number of oocytes retrieved, and number of embryos transferred (Table I).

Of the 80 patients with a positive pregnancy test, 69 progressed to a viable ongoing pregnancy (beyond 20 weeks), five underwent a clinical miscarriage (pregnancy loss after fetal cardiac activity was confirmed) and six had a biochemical pregnancy (no fetal cardiac activity confirmed).

No differences were found in the mean values of the cytokines on the basis of the etiology of infertility. IL-1α was only detected in one patient's serum.

Table II illustrates the relation between IL-14β and IL-1ra with embryo quality and pregnancy outcome. Relative antagonism of the IL-1 cytokines was positively associated with in vitro embryonic development and IVF outcome. Detectable levels of IL-1β were negatively associated with in vitro embryonic development and IVF outcome. Conversely, lower levels of IL-1ra were negatively associated in vitro embryonic development and IVF outcome.

Table I. Characteristics of Patients Analyzed for Maternal Serum Presence of IL-1 Cytokines

	($M \pm$ SD)	
	Pregnant ($n = 80$)	Not pregnant ($n = 80$)
Age	33.2 ± 2.1	33.0 ± 2.2
Previous stimulations	1.3 ± 0.5	1.3 ± 0.3
# Oocytes	12.6 ± 4.3	13.0 ± 4.6
E2 level	1222 ± 121	1262 ± 117
# ET	3.2 ± 1.1	3.3 ± 1.2

Table II. Relation Between IL-1β and IL-1ra with Embryo Quality and Pregnancy Outcome

	IL-1β			IL-1ra		
	>10 pg/mL	<10 pg/mL	*P* value	>900 pg/mL	<900 pg/mL	*P* value
High quality embryos[a]	2/22 (9%)	69/125 (55.2%)	0.001	36/51 (70.6%)	48/109 (47%)	0.003
Clinical pregnancy[b]	2/22 (9%)	61/125 (48.8%)	0.001	33/51 (64.7%)	41/109 (37.6%)	0.003

[a] High quality embryos—grade 1 or 2 out of scale grades 1–5 (1 = best; 5 = worst).
[b] Clinical pregnancy—presence of fetal cardiac activity.

DISCUSSION

This study suggests a role for the IL-1 cytokines in early pregnancy. The presence of the IL-1 cytokines in maternal serum, when utilized to supplement the synthetic medium for in vitro early embryo growth, was strongly associated with outcome. Relative antagonism of the IL-1 system was associated with higher quality embryos and higher clinical pregnancy rates. This would suggest that the IL-1 cytokines may play an important role at the very early stage of development of the embryo (1–8 cell). On the other hand, perhaps the relative antagonism of the IL-1 system was just a marker for the health of the oocytes utilized for IVF.

We have also previously demonstrated the importance of IL-1 production by endometrial cells in IVF when utilizing autologous endometrial coculture (8). We have shown that the IL-1 cytokines are elaborated in our autologous endometrial coculture system. There was an overall clinical pregnancy rate of 41.4% when embryos from patients with a history of multiple IVF failures were grown on AECC. In this study, antagonism of the IL-1 system in our coculture system was associated with IVF outcome.

Del los Santos *et al.* have also found that antagonism of IL-1 activity by IL-1ra in early embryo development appears to be beneficial (9). Del los Santos demonstrated in a single embryo that developed into a blastocyst that antagonism of the IL-1 system occurred with development of the embryo (i.e., the IL-1α+IL-1β/IL-1ra decreased as the embryo progressed from the 4-cell to the blastocyst stage). They also demonstrated that the elaboration of IL-1 cytokines required the presence of coculture with either endometrial epithelium or conditioned media from endometrial epithelium. This suggested an obligatory role of the endometrium in regulating the embryonic IL-1 system.

In contradistinction to our findings of a positive correlation with IL-1ra, in mice, high levels of exogenous IL-1ra have been shown to block implantation (6). This may reflect a varying concentration of the antagonist: agonist or may reflect a differing function of the IL-1 system at a later point in embryonic development.

CONCLUSION

The IL-1 cytokines are intimately involved with early embryo development. Relative IL-1 antagonism in maternal serum utilized to grow embryos was associated with the quality of the embryos and pregnancy outcome. This study further demonstrates the importance of the IL-1 cytokines in early embryo development.

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