Interleukin-1β, Interleukin-6, and Growth Hormone Levels in Human Follicular Fluid

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Purpose: To investigate possible relationships of interleukin-1β (IL-1β), interleukin-6 (IL-6), and growth hormone (GH) with biochemical variables in human follicular fluid (FF) and selected in vitro fertilization (IVF) parameters.

Methods: A total of 67 FF samples (n = 67 patients undergoing oocyte retrieval for IVF) was evaluated. IL-1β, IL-6, GH, hLH, FSH, PRL, hCG, testosterone, total protein, fibrinogen, sialic acid, α-antitrypsin, plasminogen levels, and spectrophotometric absorbance at 458 nm were analyzed for selected FF. IL-6 and GH levels of serum and FF samples were also compared (n = 23).

Results: Immunoreactive levels of IL-1β, IL-6, and GH were detected in all FF samples. A positive correlation existed for IL-6 (r = 0.5069, P = 0.0161) when serum-to-FF levels were compared (concentration ratio, 1:1.857). Smaller-volume follicles (<4 ml) were associated with high IL-1β levels (P = 0.0229), and an additional tendency of IL-1β to decrease with increasing embryo cleavage and scoring was observed. With the exception of a weak positive correlation between follicular IL-1β and testosterone levels (r = 0.3128, P = 0.025), no other relationship with biochemical variables or IVF parameters (etiology, e.g., endometriosis) could be implicated.

Conclusions: Substantially higher IL-6 levels occurred in FF compared to serum, thus supporting intrafollicular production. Interleukin-1β, IL-6, and GH levels in FF are, however, unsuitable markers for in vitro fertilization outcome.

KEY WORDS: human follicular fluid; growth hormone; interleukin-1β; interleukin-6; in vitro fertilization.

INTRODUCTION

Communication within the immune system occurs via soluble mediators called cytokines, which include a battery of lymphokines and monokines (1,2). These substances are produced by lymphocytes and monocytes–macrophages as well as a variety of cells (platelets, fibroblasts, keratinocytes, endothelial cells) and act on numerous cell types (1,3). The term interleukin-1 (IL-1) was introduced in 1979 to describe a single monokine which was involved in the initial activation of T cells (4). IL-6 and IL-1 are possibly the best examples of cytokines that mediate various host responses and regulate multiple cell types (2).

There is convincing evidence that cytokines play a role in two angiogenic processes, folliculogenesis and formation of the maternal decidua during early postimplantation development (5). It has been postulated that cytokines and growth factors are modulators during folliculogenesis by regulation of cell proliferation (6–8). Recent reports also suggest a potential autocrine or paracrine role for cytokines within the follicular microenvironment (9), acting as possible mediators in regulation of maternal immune cells, corpus luteum rescue, and embryo growth, quality, and development (10).

Growth hormone (GH) is secreted episodically by the anterior pituitary gland (11). Augmentation of ovarian response by exogenous biosynthetic GH or GH-releasing hormone to human menopausal gonadotropin (hMG) during ovulation induction has been demonstrated during clinical trials (12–14). A consistent body of evidence suggests that GH, and consequently insulin-like growth factor-I (IGF-I), plays a fundamental role in the regulation of follicle growth and oocyte maturation through modulatory...
actions on granulosa cell proliferation, differentiation, and steroidogenesis (15–19).

Ovulation induction during assisted reproduction give rise to follicular asynchrony. Parameters most commonly used to evaluate follicular and oocyte quality, are not adequately sensitive or specific. Thus, intrafollicular markers must be sought from within the follicle (20). Since it is known that growth hormone administration seems to augment ovarian sensitivity to gonadotropin stimulation (12–14), and cytokines are implicated as possible modulators during folliculogenesis (7–10), it is therefore important to evaluate the potential role of follicular fluid (FF) GH and proinflammatory cytokines, such as IL-1β and IL-6, as markers during assisted reproduction. The purpose of this investigation was to ascertain IL-1β, IL-6, and GH levels in blood or medium uncontaminated FF. Relationships of these cytokines as well as GH levels with (i) other biochemical variables in FF and (ii) selected in vitro fertilization (IVF) parameters were examined. Possible correlations between IL-6 and GH levels in FF and serum were additionally analyzed.

MATERIALS AND METHODS

IVF

Individual ovarian follicles were laparoscopically aspirated from women (n = 67) participating in an IVF program at the Centre for Fertility Studies, University of Pretoria. A standardized protocol for ovulation induction, oocyte retrieval and assessment, embryo culture, and evaluation, as well as the transfer technique used, has been described elsewhere (21,22). Embryo scoring was determined immediately before transfer, by multiplying the number of blastomeres of each embryo with its morphological grading (23,24). Data of patients who were successfully aspirated and had embryo transfers were included in this study.

Etiologic factors for infertility included 53.73% (36/67) tubal obstructions, 28.36% (19/67) mild endometriosis, 10.45% (7/67) idiopathic infertility (control group), and 7.46% (5/67) patients with immunological factors. The control group consisted of patients without any indications of ovarian or pelvic inflammatory diseases. Due to insufficient numbers, patients with immunological factors could not be compared with previously mentioned etiological groups. None of the patient’s husbands had an ap-

parent male factor (semen volume, ≥2.0 ml; total sperm concentration in semen, ≥40 x 10⁶/ml; motility, >70%; morphology, >14%).

FF and Serum

Following the retrieval of an oocyte and FF volume assessment, fluids were centrifuged (800g for 10 min). The cell-free supernatants were subsequently aspirated and scanned (25) with a Phillips Pye Unicam SP800 spectrophotometer. The fluids were thereafter aliquoted into 1-ml plastic cryotubes (Nunc; cat. No. 375353, Weil Organisation, RSA) and stored at −70°C until assayed. Coinciding with laparoscopy, venous blood was collected (n = 23 patients) from patients before induction of anaesthesia. The blood was centrifuged and the serum stored as previously described.

Retrospective biochemical analysis of selected preovulatory FF (n = 67) was based on spectrophotometric evaluation occluding blood- or medium-contaminated fluid. FF IL-1β, IL-6 and GH levels were correlated with fluid volume, presence of a single oocyte in the fluid (metaphase I) or lack thereof, cleavage, embryo scoring, and eventual pregnancy outcome of patients. Selection of FF in the latter category was based on the presence of a single oocyte which subsequently fertilized, cleaved, and was transferred, and a pregnancy established (defined as live birth at term).

Biochemical Analysis

All biochemical substances in FF were measured in duplicate.

(a) Interleukin-1β (n = 51), IL-6 (n = 67), and GH (n = 67) were measured using ELISA assays (Eurogenetics, Tessenderlo, Belgium): IL-1β (cat. No. E04-18-1130), IL-6 (cat. No. E04-18-1240), and GH (cat. No. E05-32-1210).

(b) Hormones were analyzed by time-resolved immunofluorometric assays (DELFIA; Pharmacia, Medical Specialities, RSA): human chorionic gonadotropin (hCG; n = 64; cat. No. 1244-007), testosterone (n = 66; cat. No. 1244-026), human luteinizing hormone (hLH; n = 65; hLH specific kit, cat. No. 1244-031), follicle stimulating hormone (FSH; n = 62; cat. No. 1244-017), and prolactin (PRL; n = 65; cat. No. 1244-018).