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Physiology

Insulin-like growth factor-1 and insulin-like growth factor binding protein-1 and 3 in the follicular fluid of infertile patients submitted to in vitro fertilization

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***Purpose*:** In the present article we propose to evaluate IGF-1, IGFBP-1 and 3 in the follicular fluid of infertile patients submitted to in vitro fertilization.
***Methods*:** We performed a case-control study with 53 infertile patients submitted to the first in vitro fertilization attempt. We compared their follicular fluid concentration of IGF-1, IGFBP-1 and IGFBP-3 between the patients who became pregnant ($n = 11$) versus those nonpregnant ($n = 42$).
***Results*:** The clinical characteristics of patients from the two groups were similar in terms of age and body mass index. Data related to the analysis of ovulation induction was not different regarding length of induction in days, number of retrieved oocytes, fertilization rate, and number of transferred embryos. Furthermore, the number of FSH units required for ovarian induction was also similar between the studied groups.

IGF-1 and IGFBP-1 were not significantly different between the groups ($p > 0.05$). However, those patients that became pregnant presented a lower follicular fluid concentration of IGFBP-3, 2237.10 ± 582.73 pg/ml and 2657.64 ± 584.15 ng/ml, respectively ($p = 0.038$).
***Conclusions*:** We demonstrated an association of a lower follicular fluid IGFBP-3 in individuals that became pregnant compared to subjects that did not after in vitro fertilization.

KEY WORDS: IGF-1; IGFBP-1; IGFBP-3; In vitro fertilization; Follicular fluid.

INTRODUCTION

The assessment of follicular fluid milieu is very important to understand and even modulate human ovulation. Increased concentrations of estradiol/androstenedione were described when mature oocytes are present. Moreover, the regulatory and amplificatory effect of follicular fluid growth factors were evaluated in different groups of patients and showed to be fundamentally important for an adequate ovarian function (1).

In the mammalian ovaries, insulin-like growth factor-1 (IGF-1) seems to have an autocrine/paracrine important action. This peptide is produced by granulosa cells after FSH stimulation, and its activity is controlled by catalytic proteins and by insulin-like growth factor binding proteins (IGFBP), which act as inhibitors or regulators of its action (2). In addition, IGF-1 activates the production of estrogen and progesterone and the activity of aromatase, and also modulates the effect of FSH and LH (3,4).

Furthermore, the ovulation control exerted by IGF-1 is evidenced by the fact that in induced assisted reproduction technology (ART) cycles, follicles with higher IGF-1 concentrations require a lower number of FSH ampoules and a shorter induction time (5).

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On the other hand, IGFBP-1 exerts an inhibitory effect on IGF-1, acting as an antigonadotrophic agent (6,7) and modulating IGF-1 activity. IGFBP-3 in follicular fluid could exert also a regulatory role, serving as an inhibitory effect on IGF-1 and 2 (8–10). Its increased concentration may decrease IGF-1 and 2 availability on follicular fluid and, consequently, its action.

Some authors (5,11) investigated the correlation of IGFBP-3 and ART demonstrating that IGF-1 and IGFBP-3 were associated with oocyte maturation and its follicular fluid concentration were higher in patients receiving FSH highly purified than hMG. Moreover, IGFBP-3 follicular fluid concentration in older women did not differ from younger controls (12).

However, this correlation is disputed by others (13) who showed that IGFBP-3 was not associated with any IVF outcome. These differences could be related to the diverse controlled ovarian stimulation protocols and studied populations.

With the aim of elucidating the role of some important growth factors on oocyte development and IVF outcome (pregnancy rate), we decided to analyze the follicular fluid environment of infertile patients submitted to IVF through the analysis of IGF-1, IGFBP-1, and IGFBP-3 concentrations.

MATERIALS AND METHODS

Design

A case-control study was performed in order to analyze the follicular fluid infertile patients submitted to IVF.

Patients

A total of 53 patients were studied, we divided those patients in two groups, according the primary IVF outcome (clinical pregnancy). Group I (cases) was comprised by 11 patients that after IVF became pregnant and, group II (controls) was formed by 42 patients that after the first IVF attempt did not get pregnant.

IGF-1, IGFBP-1, and IGFBP-3 concentrations were assessed in the follicular fluid of all 53 infertile patients submitted to IVF (first cycle) as the treatment for infertility at the Human Reproduction Unit, Hospital de Clínicas de Porto Alegre, Brazil, between January 2001 and January 2002. Patients were informed about the procedures and spontaneously signed an informed consent form. The research project was approved by the Ethics Committee and registered at the Graduate Research Group of the hospital.

The following inclusion criteria were established: 1) patients with no previous endocrine disorders; 2) cases in which the cause for infertility was only tubal obstruction; 3) patients with serum FSH dosage lower than 10 IU/mL in the early follicular phase (3rd day of menstrual cycle). The criteria proposed by the World Health Organization (1992) were used for the analysis of sperm.

In Vitro Fertilization

IVF was performed for the treatment of infertility due to the presence of tubal obstruction. The ultrashort protocol was used for ovulation induction, with GnRH analogue.

Briefly, on the 3rd day of the first menstrual cycle after the beginning of the study protocol, recombinant FSH was administered at the initial dose of 150 IU, with dose adjustment according to patient response. Patients received leuprolide acetate from the second to the 4th day of the menstrual cycle (1 mg/day subcutaneously).

Ovulation induction was monitored by daily ultrasonography. When at least one ovarian follicle had reached 16 mm diameter, hCG at the dose of 10.000 IU was administered intramuscularly, and oocyte collection occurred from 34 to 36 h afterwards.

Samples of follicular fluid, measuring more than 15 mm (15–18), in which an oocyte-cumulus complex was found were centrifuged at 2500 rpm and frozen at $-20^{\circ}C$ for posterior analysis, we used only clear (without blood) fluid. In order to homogenate the analysis, we pooled a small sample of follicular fluid of all follicles with similar (15–18) volumes for posterior analysis.

Serum FSH levels (day 3) were analyzed using chemiluminescence kits (Immulite Ltd., USA); with the largest inter- and intra-kit variation was 8.1 and 7.7%, respectively.

Follicular fluid (IGF-1, IGFBP-1 and 3) analysis was performed at the radioimmunoassay laboratory of Hospital de Clínicas the Porto Alegre, Brazil, with specific kits (DSL, Texas, USA). The result was obtained through the two-site immunoradiometric assay (IRMA). IRMA is a highly sensitive and specific assay method that uses the competition between radios labeled and unlabeled substances in an

antigen-antibody reaction to determine the concentration of the unlabeled substance.

The minimal detection limit of IRMA was 0.8 ng/ml (corrected to 1:30 dilution) or 27 pg/ml (1.4 pg/tube) for IGF-1, 0.33 ng/ml for IGFBP-1, and approximately 0.5 ng/ml for IGFBP-3. The coefficient of variation was 1.5% for IGF-1, 2.7% for IGFBP-1, and 0.5% for IGFBP-3.

Statistics Analysis

We compared the groups using *t*-test for the analysis of continuous data. Otherwise, categorical data were analyzed using Fisher's exact test. The sample size was calculated (power of 80%) to compare differences regarding IGF-1, IGFBP-1 and IGFBP-3) levels in the follicular fluid ($SD = 580$ and a difference between the means of 500. The expected precision was (95% C.I.) observed difference between means, plus or minus 351.7. For these parameters, the sample size was 46 patients. Moreover, the analysis was considered significant when $p < 0.05$.

RESULTS

The clinical characteristics of patients from the two groups are presented in Table I and comparable in terms of age and body mass index (BMI). This table also presents the IVF characteristics which could interfere in our results.

Table I. Clinical and IVF Patients' Characteristics (Mean and *SD*)

	Cases ($n = 11$)	Controls ($n = 42$)	Statistics (p)
Age (years)	31.9 ± 3.4	31.4 ± 4.7	0.714
BMI (Kg/m^2)	22.7 ± 2.6	23.1 ± 2.6	0.706
Length of stimulation (days)	8.2 ± 1.0	8.7 ± 2.1	0.382
FSH (IU) administered	733 ± 213	628 ± 338	0.316
Number of retrieved oocytes	4.4 ± 2.8	4.0 ± 3.2	0.686
Fertilization rate	77%	70%	0.498
Number of embryos	2.0 ± 1.1	1.9 ± 2.1	0.854
Transferred embryos	1.7 ± 0.9	1.5 ± 1.4	0.696

Data related to the analysis of ovulation induction is very similar between both groups of patients; we did not identified differences regarding length of induction in days, number of retrieved oocytes, fertilization rate, and number of transferred embryos. Furthermore, the number of FSH units required for ovarian induction was also similar between the studied groups.

Follicular fluid growth factor levels are shown in Fig. 1. IGF-1 and IGFBP-1 were not significantly different between the groups ($p > 0.05$). However,

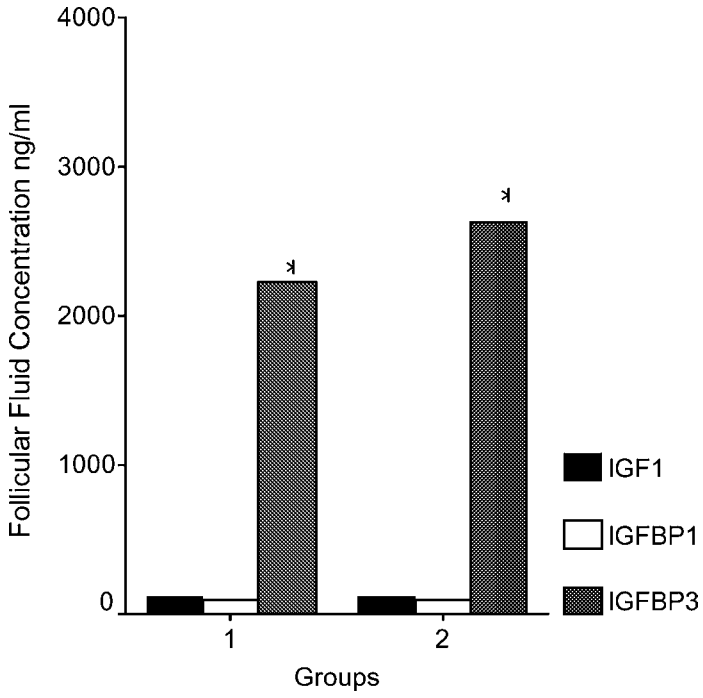

Fig. 1. Follicular fluid (mean) of IGF-1, IGFBP-1 and 3, in Group 1 (cases) and 2 (controls).*$p = 0.038$, *t*-test.

those patients that became pregnant after the first IVF attempt presented lower follicular fluid concentrations of IGFBP-3 than those non-pregnant patients; 2237.10 ± 582.73 pg/mL and 2657.64 ± 584.15 ng/mL, respectively ($p = 0.038$).

All included follicles measured 15–18 mm, and the Levene's test for homogeneity was not significant ($p > 0.05$).

DISCUSSION

We demonstrated that IGFBP-3 could be associated with a good reproductive outcome after IVF using the ultra-short protocol for controlled ovarian stimulation (COS). Moreover, IGF-1 and its binding protein (IGFBP-1) were not different between those patients who became pregnant or not after the first IVF attempt. Furthermore, the follicular cohort was very homogeneous (15–18 mm) which did not interfere our results and conclusions.

Our results are in agreement with Amato *et al*., 1998 (8) who showed the reduction of IGFBP-3 in follicular fluid after COS, this result could cooperate on IGF-1 and 2 amplification, increasing its stimulatory effect on follicular development. However, the follicular growth and maturation, during the FSH-dependent phase, is related to several peptides, growth factors, hormones and modulators.

Furthermore, this association between IGFBP-3 and follicular growth or reproductive chance is disputed by others (5,12).

Poor responders patients compared to good responders could have the same follicular fluid concentration of IGFBP-3; in addition, these authors (5) did not find any correlation in terms of IGFBP-3 in follicular fluid and IVF outcomes. Conversely, patients who have received highly purified FSH during their COS presented higher follicular IGFBP-3 fluid concentration than those patients whose hMG was administered in COS.

This finding was also demonstrated by others that the administration of recombinant FSH could affect the follicular fluid concentration of IGFBP-3 However, the authors did not associate this peptide with another IVF outcome (12).

The differences in terms of results presented by these investigators could be explained by the fact of design, patient selection (studied population), inclusion criteria, and reproductive outcome chosen and, also, the controlled stimulation protocol utilized.

We decided to include and analyze only patients with tubal obstruction as the infertility etiology. Moreover, our research differs from the others by the fact that we performed the ovarian hyoerstimulation with the ultra-short protocol.

Our research emphasizes the importance of these peptides on ovarian response after COS and that a good and competent oocyte, measured by IGFBP-3, is more prone to develop a good quality and capable embryo. We can explain our findings by the fact that decreasing the IGFBP-3 content in follicular fluid the IGF-1 availability could be increased, consequently, the free content of this peptide may favors oocyte development and competence, as demonstrated by others (9).

Nevertheless, we can not simply conclude that pregnancy after COS is merely associated with the follicular content of IGFBP-3 or any studied growth factor or binding protein. There are several well-studied factors (semen, maternal age, infertility length) which are well correlated to the IVF success.

In addition, there are several growth factors and peptides which could interfere with the follicular development, oocyte competence and ART outcomes.

In conclusion, we showed that IGF-1 and IGFBP-1 were not associated with pregnancy after IVF. On the other hand, follicular fluid content of IGFBP-3 during COS was lower in pregnant patients after the first IVF attempt. We emphasize that our results are only applicable in patients submitted to IVF with the ultra-short stimulation protocol and more research is needed concerning the effect of these growth factors in others COS protocols (long and GnRH antagonist protocols).

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