Inhibin B Predicts Oocyte Number and the Ratio IGF-I/IGFBP-1 May Indicate Oocyte Quality During Ovarian Hyperstimulation for In Vitro Fertilization

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Purpose: To perform a retrospective analysis of 62 age-matched IVF-treated women in order to investigate whether levels of inhibin B, IGF-I, and IGFBP-1 in serum 2 days before oocyte retrieval and in follicular fluid at the day of oocyte retrieval might be useful as indicators of the ovarian ability to produce oocytes (ovarian reserve).

Methods: Patients were allocated into three groups on the basis of the number of oocytes retrieved. Group 1 ("low responders") had 0–3 oocytes, group 2 ("normal responders") had 6–11 oocytes, and group 3 ("high responders") had 12 oocytes or more. Levels of inhibin B, IGF-I, and IGFBP-1 in follicular fluid and in serum obtained 2 days before oocyte retrieval were analyzed and correlated to clinical parameters including estradiol levels, progesterone levels, follicle size, follicle number, and oocyte number.

Results: We found significant differences in inhibin B levels in the three groups. Inhibin B levels in follicular fluid and serum was strongly correlated to the number of oocytes retrieved ($p < 0.01$). The number of oocytes retrieved were also correlated to total FSH dose ($p < 0.05$), to estradiol 2 days before and at ovum pick-up ($p < 0.05$), to progesterone at ovum pick-up ($p < 0.0001$), to progesterone at embryo transfer ($p < 0.05$), and to the number of follicles (size 12–15 mm, $p < 0.001$, size > 15 mm, $p < 0.01$). Serum inhibin B also correlated to follicular fluid inhibin B ($p < 0.01$). Inhibin B was not correlated to pregnancy. In contrast, the ratio IGF-I/IGFBP-1 in serum as well as in follicular fluid was significantly higher in women who became pregnant ($p < 0.05$).

Conclusions: The results show that inhibin B in serum 2 days before oocyte retrieval predicts number of oocytes retrieved. Since inhibin B in serum before oocyte retrieval in ovarian hyperstimulation was strongly predictive of the number of oocytes retrieved, it appears useful as a marker for ovarian response. Inhibin B did not predict treatment outcome, whereas the ratio IGF-I/IGFBP-1 in serum and follicular fluid was significantly higher in women who became pregnant. The ratio IGF-I/IGFBP-1 may thus reflect oocyte quality.

KEY WORDS: IGF-I; IGFBP-1; inhibin; IVF; oocyte.

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INTRODUCTION

In vitro fertilization (IVF) has become an established standard method for the treatment of infertility. A key factor in this process is the capacity of the ovary to produce good quality oocytes. Therefore, it is important to be able to assess ovarian function before treatment. This serves both in order to be able to adjust the treatment protocol, and in counselling the patients about reasonable expectations of treatment outcome. The search for predictive factors in IVF treatment has so far shown that the most useful test is the clomiphene citrate challenge test (CC-test), which consists of an assay of basal serum follicle stimulating hormone (FSH) on cycle day 3, and again on cycle day 10 after administration of 100 mg clomiphene citrate cycle days from 5 to 9 (1).

In the search for predictive factors for treatment outcome, attention has been given to growth factors that have been believed or shown to influence ovarian follicle growth, see e.g. (2). Factors that have been examined in this context include the insulin-like growth factors I and II (IGF-I and IGF-II) together with their binding proteins, the insulin-like growth factor binding proteins (IGFBPs) and the inhibins (3,4).

The inhibins are small peptides belonging to the transforming growth factor beta-family. Physiologically active inhibins exist as disulphide-linked dimers, consisting of one alpha-subunit and one beta-subunit. There are two types of beta-subunits, beta-A and beta-B, and the alpha/beta-A combination is called inhibin A whereas the alpha/beta-B is called inhibin B (5,6).

Studies of inhibins across the menstrual cycle indicate that inhibin B concentrations rise from the luteal phase to the follicular phase, with maximal levels in the midfollicular phase. This indicates that inhibin B is secreted from the developing cohort of follicles in the cycle (7,8). Inhibin A concentrations begin to rise after the increase of serum estradiol (E2) in the late follicular phase, which suggests secretion by the dominant follicle only (9–12). Thus, it has been suggested that inhibin B could indicate the number or quality of developing follicles (13), whereas inhibin A may indicate follicle maturity (14).

Previous studies have examined whether inhibin A and B may be useful as predictive factors in assisted reproduction (15–18). The results indicate that both inhibins A and B reflect ovarian function, but are less useful in predicting stimulation outcome (17), and a comparison of the clomiphene citrate challenge test with inhibin B testing on cycle day 3 and 10 showed no clinical value of inhibin B testing (16).

Previous studies of IGFs and IGF-binding proteins in the ovary are conflicting. IGFs in serum are bound to binding proteins, mainly IGFBP-3 and IGFBP-2 (19). The free levels of IGF-I range from 0.5 to 5% of total IGF levels. IGF-I levels in ovarian follicle fluid is usually correlated to serum levels, and since presence of IGF-I mRNA in granulosa cells is controversial (20), it has been suggested that IGF-I in follicular fluid may largely be present as a result of transudation from serum (21, 22) and may not be essential for ovarian follicular development (23). Still, there are studies reporting that follicular fluid IGF-I vary as a function of ovarian reserve and gonadotropin stimulation (24).

Rapid variations in the serum concentration of IGF-I are regulated by changes in levels of IGFBP-1. IGFBP-1 mRNA has been shown to be present in granulosa cells of dominant follicles (25). IGFBP-1 is also increased in luteinizing follicles (22) and in follicles containing mature oocytes (26), suggesting a role for IGFBP-1 in the development of the dominant follicle and in corpus luteum regulation, and also its use as a marker for mature oocytes (26). Investigations of the free fractions of IGFs in different clinical situations have indicated that free levels of IGF-I decrease during situations of stress or catabolism (27). Thus, availability of IGF-I, as a function of the levels of IGF-I in relation to levels of IGFBP-1 (i.e. the ratio between IGF-I and IGFBP-1) may be useful as a marker for biological response of the IGF-I system in various treatments.

Inhibin B in the previous studies, although not predictive of outcome, appeared to be correlated ovarian function and the developing cohort of follicles. We therefore hypothesized that inhibin B might be useful as an indicator of the ability of the ovary to produce oocytes, also called ovarian reserve, and therefore might be correlated to ovarian response after stimulation. We have, in the present study, performed a retrospective analysis of inhibin B, IGF-I, and IGFBP-1 in 62 women that underwent ovarian stimulation for IVF in our center. Age-matched patients were allocated into three groups on the basis of the number of oocytes retrieved. Group 1 (“low responders”) had 0–3 oocytes, group 2 (“normal responders”) had 6–11 oocytes, and group 3 (“high responders”) had 12 oocytes or more. Inhibin B, IGF-I, and IGFBP-1 were analyzed in serum obtained 2 days before oocyte retrieval and in follicular fluid obtained at oocyte retrieval. Levels of inhibin B, IGF-I, and IGFBP-1 were then correlated to a number of clinical parameters including estradiol levels, progesterone levels, follicle number, and oocyte number.

MATERIAL AND METHODS

Study Population

The study population consisted of 62 patients treated in our clinic in 1996–97, and encompasses a total of 62 cycles. During this time, criteria for admission to our IVF programme was age <38 years, no previous children with the current partner, and presence of $>1 \times 10^6$ progressively motile spermatozoa/mL after swim-up preparation (intracytoplasmic sperm injection (ICSI) was not performed in our center at this time). The study was approved by the local ethics committee as a part of a larger study of epidemiological and biological predictors of IVF success.

Patient information collected included age, infertility diagnosis, and number of previous IVF attempts. Information regarding stimulation characteristics and outcome included FSH starting dose and total dose, stimulation length (i.e. the day from the first injection to the day of ovum pick-up (OPU), sperm count, and number of oocytes obtained. The average age of patients was close to 33 years, and most of them had done two previous IVF cycles. Reasons for infertility included tubal factor, endometriosis, male factor, mixed factors, and unexplained infertility. Characteristics of the patients in the study are given in Table I.

Blood samples were collected in the fasting state at the day of human chorionic gonadotropin (hCG) administration, and assayed for inhibin B, IGF-I, IGFBP-1, estradiol, and progesterone. 17β -Estradiol (E2) was also analyzed at the start of stimulation, before oocyte retrieval (also called ovum pick-up (OPU)), and at embryo transfer (ET). In order to obtain representative follicular fluid from mature follicles containing mature oocytes, follicular fluid was obtained as a pool from the three first, and largest, follicles aspirated, and was assayed for inhibin B, IGF-I, and IGFBP-1. The aspirates were not diluted with saline wash.

Ovarian Stimulation Protocol

All patients followed our standard long GnRHa-FSH protocol for stimulation as described earlier (28,29). Gonadotropin releasing hormone agonist (GnRHa) (Suprefact, Hoechst AB, Stockholm, Sweden) was administered as a nasal spray $6 \times 200 \mu\text{g/day}$ starting on day 21 of the menstrual cycle, and was reduced to half the dose when FSH injections were commenced. Downregulation was verified by

vaginal ultrasound scanning, followed by administration of 75–300 IU/day of recombinant FSH (Gonal-F, Serono Nordic AB, Sollentuna, Sweden; Puregon, Organon Sweden AB, Göteborg, Sweden) subcutaneously (s.c.).

Starting dose was adjusted according to the same rules for all patients. The initial dose of FSH was generally 150 IU up to age 35, and 225 IU above this age, unless response to previous FSH stimulation at IVF indicated otherwise. In cases with the diagnosis of polycystic ovaries and a known tendency for strong response to FSH stimulation, the starting dose was 75–100 IU. Previously known low responders were given 300 IU FSH.

Follicular development and endometrial growth were monitored by vaginal ultrasonography using a Siemens Sonoline SI/200 in combination with blood samples for E2 assays. When an adequate stimulation was achieved, i.e. a controlled rise of serum estradiol and a leading follicle diameter of at least 17 mm, 10,000 IU hCG (Profasi, Serono Nordic AB, Sollentuna, Sweden) was given s.c. Approximately 35 h later OPU was performed by transvaginal ultrasound-guided follicle aspiration. IVF, ET, and pregnancy follow-up was performed as described elsewhere (28). Luteal phase support was given using 400 mg micronized progesterone as vaginal suppositories 3 times daily until a pregnancy test was performed and if found positive, continued 8 weeks after embryo transfer. Pregnancy was defined as a serum hCG level $>10 \text{ IU/L}$ 2 weeks after ET and subsequently rising. Clinical pregnancy was defined as presence of at least one intrauterine fetus with regular heart beats. Criteria for cycle cancellation were (a) no rise in estradiol even after an increase in dose, (b) three or fewer follicles approaching 17 mm diameter, (c) high risk for developing ovarian hyperstimulation syndrome (OHSS)—sharp rise in E2 and >25 follicles.

Hormone Assays

IGF-I was determined by radioimmunoassay (RIA) as described previously using acid ethanol extraction (30,31). The sensitivity of the assay was 0.02 ng/mL and the intra- and interassay coefficients of variation were 4 and 11%, respectively. IGFBP-1 was determined by radioimmunoassay, as described previously (32), with a sensitivity of $3 \mu\text{g/L}$ and intra- and interassay coefficients of variation of 3 and 11%, respectively.

Inhibin B in follicular fluid and serum was determined using a two-step immunoassay (MCA1312KZZ)

according to the instructions from the manufacturer (Serotec, Oxford, U.K.). The sensitivity of the inhibin B assay was <15 pg/mL. The intra- and interassay coefficients of variation were both <7%.

Serum concentrations of FSH were assayed by RIA from the Farnos Group (Oulu, Finland) and by the Central Laboratory for Clinical Chemistry, Karolinska Hospital. The assay is standardized both by the World Health Organization's Second International Reference preparation of FSH for Bioassay, number 78/549, and by the WHO 2nd IRP-HMG. The sensitivity of the FSH assay was 0.7 mIU/ml. The intra- and interassay coefficients of variation were 5.4 and 4.9%, respectively. Our upper level for normal FSH is 10 IU/L, calibrated to World Health Organization standard 78/549. This cutoff level is widely used in Europe (for premenopausal women in the early follicular phase the normal range, 3.4–10 IU/L), and corresponds to a level of 17 IU/L if the assay is calibrated against the Second IRP standard, widely used in the United States. We presently regard women with FSH levels equal to or below 10 IU/L as having a normal ovarian reserve, whereas women with FSH levels exceeding 10 IU/L are regarded to have a diminished ovarian reserve.

E2 and progesterone (P4) levels were determined by RIA from the Diagnostic Products Corp., Los Angeles. The intra- and interassay coefficients of variation were 7.2 and 6.8%, and 4.9 and 5.8%, respectively.

Statistical Analysis

Prior to statistical analysis, data were tested for Gaussian distribution using a normality test (the Kolmogorov–Smirnov test). Some data were found not to be normally distributed. Therefore, nonparametric statistics were used for all the data. Comparisons between mean values were made using the Mann–Whitney Rank Sum Test. Although the standard errors were not, therefore, used for statistical comparisons, they have been appended to the mean values in the tables. Correlation analysis was performed using Spearman correlation. Tests were performed with the statistical package Graphpad Prism and Statmate (Graphpad Software Inc, San Diego, CA). A *p* value <0.05 was considered significant.

RESULTS

Study Population

The patients were grouped according to number of oocytes retrieved at OPU. There was no significant difference between the groups regarding age, duration of stimulation, sperm count of the partner or endometrial thickness before OPU (Table I). There were, however, differences regarding starting dose and total dose of FSH used, which was higher in the group with low number of oocytes retrieved.

Table I. Clinical Characteristics of the Study Population and the Stimulation Cycle. Differences Between the Groups Occurred Except Where Indicated by ns. (Values given as mean $\pm$ SD where not otherwise indicated)

Characteristic	Low (<i>n</i> = 21)	Normal (<i>n</i> = 23)	High (<i>n</i> = 18)	
Age	32.8 $\pm$ 0.8	32.9 $\pm$ 0.7	32.8 $\pm$ 0.8	n.s.
Number of previous cycles	2.04 $\pm$ 0.97	1.74 $\pm$ 0.91	2.22 $\pm$ 1.22	
Starting dose of FSH (IU)	220 $\pm$ 24	173 $\pm$ 10	201 $\pm$ 9	
Total dose of FSH (IU)	2798 $\pm$ 308	1983 $\pm$ 131	2086 $\pm$ 100	
Duration of stimulation (d)	15.38 $\pm$ 0.55	15.04 $\pm$ 0.46	14.33 $\pm$ 0.25	n.s.
Sperm count ($\times 10^6$)	5.84 $\pm$ 0.67	5.77 $\pm$ 0.55	5.63 $\pm$ 1.02	n.s.
Endometrium (mm)	11.3 $\pm$ 0.7	11.1 $\pm$ 0.5	11.8 $\pm$ 0.5	n.s.
Number follicles 12–15 mm	4.5 $\pm$ 1.2	8.7 $\pm$ 1.8	10.9 $\pm$ 1.2	
Number follicles >15 mm	4.7 $\pm$ 1.13	6.8 $\pm$ 0.8	9.9 $\pm$ 1.5	
Number of oocytes	2.23 $\pm$ 0.70	8.22 $\pm$ 1.59	14.28 $\pm$ 2.35	
Number of fertilized oocytes	1.71 $\pm$ 0.19	5.52 $\pm$ 0.47	7.17 $\pm$ 1.15	
Number of cleaved preembryos	1.57 $\pm$ 0.22	5.05 $\pm$ 0.45	7.06 $\pm$ 1.23	
Number of preembryos transferred	1.38 $\pm$ 0.16	1.96 $\pm$ 0.04	1.67 $\pm$ 0.18	
Indication (<i>n</i>)				
Tubal	6	6	8	
Endometriosis	8	5	2	
Male	0	4	3	
Mixed	3	3	3	
Unexplained	1	6	2	

There were also differences regarding parameters related to the number of oocytes, i.e. number of follicles, number of fertilized oocytes, and number of cleaved and transferred preembryos. Finally, there were also differences in the groups regarding indication for treatment, with a higher number of patients with endometriosis in the group with lower oocyte numbers (Table I).

Relationship Between Inhibin Levels and Stimulation Parameters

Inhibin B in serum 2 days before OPU and in follicular fluid at the day of OPU was correlated to the number of oocytes retrieved (serum: $r = 0.337$; follicular fluid: $r = 0.344$, $p < 0.01$). There was a significant difference between inhibin B levels in the three groups of patients, with lowest inhibin B levels in the group with few oocytes retrieved (Fig. 1, Table II). Inhibin B in serum 2 days before OPU also correlated to inhibin B in follicular fluid at the day of OPU ($r = 0.392$, $p < 0.01$). Inhibin B levels were neither in serum nor in follicular fluid associated to treatment outcome. There was no difference in inhibin B levels in nonpregnant versus pregnant women (Table III).

Relationship Between Oocyte Number, Hormone Levels, and Follicle Size

The number of oocytes retrieved was also correlated to the total FSH dose given during the stimulation ($r = -0.289$, $p < 0.05$), to the E2 levels in serum 2 days before OPU ($r = 0.301$, $p < 0.05$), and to the E2 levels in serum at the day of OPU ($r = 0.363$, $p < 0.01$). The number of oocytes retrieved was furthermore correlated to the number of follicles observed 2–3 days before OPU (follicles between 12 and 15 mm diameter ($r = 0.430$, $p < 0.001$), follicles >15 mm diameter ($r = 0.336$, $p < 0.01$), to the levels of P4 in serum at the day of OPU ($r = 0.602$, $p < 0.0001$), and to the P4 levels in serum at the day of ET ($r = 0.279$, $p < 0.05$). E2 levels in serum on the day of OPU were, furthermore, correlated to the number of small (diameter 12–15 mm; $r = 0.405$, $p < 0.01$) and large (diameter >15 mm; $r = 0.276$, $p < 0.05$) follicles observed 2–3 days before OPU. The P4 levels in serum at the day of OPU were furthermore strongly correlated to the E2 levels in serum on the day of OPU ($r = 0.435$, $p < 0.0001$) and to the E2 levels in serum 2 days before OPU ($r = 0.305$, $p < 0.01$).

Relationship Between IGF-I and IGFBP-1 Levels to Stimulation Parameters

IGF-I levels in serum 2 days before OPU and in follicular fluid at the day of OPU were strongly correlated to each other ($r = 0.593$, $p < 0.0001$). IGFBP-1 levels in serum 2 days before OPU and in follicular fluid at the day of OPU were weakly correlated ($r = 0.295$, $p < 0.05$). The ratio of IGF-I/IGFBP-1 was positively correlated to oocyte number in serum 2 days before OPU, but not in follicular fluid at the day of OPU. The ratio of IGF-I/IGFBP-1 in both in serum at the day of OPU and in follicular fluid at the day of OPU was significantly higher in women that became pregnant as a result of the treatment as compared to the women that did not become pregnant ($p < 0.05$; see Table III).

DISCUSSION

The results show that inhibin B levels in serum obtained 2 days before OPU may predict the number of oocytes retrieved. Serum inhibin B levels 2 days before OPU were correlated to follicular fluid inhibin B at the day of OPU, which also was correlated to the number of oocytes retrieved.

These results support the use of inhibin B levels in serum early during FSH treatment as an index of the number of recruited follicles that will ultimately reach maturity (33). The ratio of IGF-I/IGFBP-1 in serum 2 days before OPU and the levels of P4 in serum at the day of OPU were also correlated to oocyte number (Table II) and these two parameters thus also appear to be useful in this regard.

Inhibin B is believed to be predominantly produced in and released from the ovarian granulosa and luteal cells (8,34,35), therefore it is logical that the levels of inhibin B were correlated to the number of oocytes and thus indirectly to the number of mature follicles. Inhibin B may therefore be regarded as a useful marker for ovarian reserve and/or ovarian response to controlled hyperstimulation. Previous studies have suggested that basal follicle-stimulating hormone and inhibin B have similar predictive properties and could therefore be used interchangeably (36).

Further studies comparing basal day 3 FSH and inhibin B have suggested that inhibin B is a stronger predictor, since women with declining ovarian responsiveness and clinical outcomes consistent with

Fig. 1. Inhibin B levels in (a) follicular fluid (ng/mL) on the day of ovum pick-up (OPU) and (b) serum (pg/mL) 2 days before OPU in three groups of women—low, normal, and high responders (related to oocyte counts after OPU) in controlled ovarian hyperstimulation for IVF (mean $\pm$ SD).

Table II. Levels of Inhibin B, IGF-I, IGFBP-1, and the Ratio IGF-I/IGFBP-1 in Serum 2 Days Prior to OPU (OPU-2) and in Follicular Fluid at the Day of ovum pickup (OPU); and Levels of E2 and P4 in Serum on the Day of OPU in Low, Normal, and High Responders After Controlled Ovarian Hyperstimulation for IVF (mean $\pm$ SD)

	Low (<i>n</i> = 21)	Normal (<i>n</i> = 23)	High (<i>n</i> = 18)	
Serum at OPU-2				
Inhibin B (pg/mL)	492 $\pm$ 103	923 $\pm$ 140	1132 $\pm$ 150	<i>p</i> < 0.01
IGF-I (ng/mL)	251 $\pm$ 20	264 $\pm$ 15	263 $\pm$ 22	ns
IGFBP-1 (ng/mL)	23 $\pm$ 4	21 $\pm$ 2	24 $\pm$ 3	ns
IGF-I/IGFBP-1	10.90 $\pm$ 4.20	12.50 $\pm$ 2.17	15.95 $\pm$ 2.65	ns
Serum at OPU				
E2 (pmol/mL)	2714 $\pm$ 288	3199 $\pm$ 310	3978 $\pm$ 348	<i>p</i> < 0.05
P4 (nmol/mL)	26.3 $\pm$ 2.6	37.6 $\pm$ 2.6	52.7 $\pm$ 5.9	<i>p</i> < 0.01
Follicle fluid at OPU				
Inhibin B (ng/mL)	198 $\pm$ 75	414 $\pm$ 59	397 $\pm$ 75	<i>p</i> > 0.05
IGF-I (ng/mL)	189 $\pm$ 9	209 $\pm$ 12	189 $\pm$ 12	ns
IGFBP-1 (ng/mL)	143 $\pm$ 12	118 $\pm$ 9	122 $\pm$ 13	ns
IGF-I/IGFBP-1	1.32-0.16	1.77 $\pm$ 0.18	1.54 $\pm$ 0.27	ns

Note. Differences Between the Low Oocyte Count Group and the Other Groups Are Indicated to the Right of the Columns.

declining ovarian reserve had decreased day 3 serum inhibin B levels despite having nonelevated day 3 serum FSH concentrations (18). Declining ovarian reserve may thus be demonstrated by a decrease in day 3 inhibin B levels before a rise in day 3 FSH levels. Inhibin B testing has been performed together with the most commonly used test for ovarian reserve, the CC-test. It was found that inhibin B was diminished in women with reduced ovarian reserve according to the CC-test (37), but there was no clinical value in inhibin B testing as compared to the CC-test as a predictor of pregnancy (16).

In IVF, the number of oocytes obtained at OPU is generally, but not always, in some respect

proportional to the number of good quality embryos obtained. In addition to a marker for oocyte number, it would be desirable to have a marker for oocyte quality. Such a marker would be expected to be correlated to pregnancy rate. In the present study, inhibin B levels neither in serum nor in follicular fluid were correlated to pregnancy, and thus we conclude that inhibin B does not appear to reflect oocyte quality. This is in line with the above mentioned study, which also found no correlation between inhibin levels and pregnancy rate (16).

In contrast to inhibin B, the present study showed that the ratio of IGF-I/IGFBP-1 both in serum and in follicular fluid was correlated to pregnancy rate. The ratio of IGF-I/IGFBP-1 in serum and follicular fluid therefore appears useful as an index of oocyte quality. Previous studies have suggested both IGF-I and IGFBP-1 as indicators of oocyte quality or oocyte maturity (26,38–40). Furthermore, several studies have shown that both exogenous and endogenous IGF-I have positive effects on preembryo development and blastocyst formation (41–46).

The ovarian contribution to the circulating levels of IGF-I in serum during ovarian hyperstimulation is likely to be very small, since no IGF-I mRNA was detected in the ovary (20). Current data suggest that most of the follicular fluid IGF-I comes from serum (21), may be with a small contribution from theca cells (47). The possibility of a local ovarian production of IGF-I is in line with our finding of a significant correlation between inhibin B and IGF-I in follicular fluid, but not in serum.

Table III. Levels of Inhibin B, IGF-I, IGFBP-1, and the Ratio IGF-I/IGFBP-1 in Serum 2 Days Prior to OPU (OPU-2) and in Follicular Fluid at the Day of OPU; and Levels of E2 and P4 Serum at the Day of OPU in Nonpregnant and Pregnant Women After Controlled Ovarian Hyperstimulation for IVF (mean $\pm$ SD)

	Nonpregnant (<i>n</i> = 46)	Pregnant (<i>n</i> = 16)	
Serum at OPU-2			
Inhibin B (pg/mL)	866 $\pm$ 615	765 $\pm$ 704	ns
IGF-I (ng/mL)	251 $\pm$ 82	284 $\pm$ 79	ns
IGFBP-1 (ng/mL)	23 $\pm$ 14	20 $\pm$ 10	ns
IGF-I/IGFBP-1	10.09 $\pm$ 2.2	14.20 $\pm$ 2.17	<i>p</i> < 0.05
Serum at OPU			
E2 (pmol/mL)	3392 $\pm$ 1609	2883 $\pm$ 1057	ns
P4 (nmol/mL)	39.23 $\pm$ 20.02	34.8 $\pm$ 17.42	ns
Follicle fluid at OPU			
Inhibin B (ng/mL)	331 $\pm$ 262	346 $\pm$ 301	ns
IGF-I (ng/mL)	191 $\pm$ 49	211 $\pm$ 56	ns
IGFBP-1 (ng/mL)	131 $\pm$ 12	119 $\pm$ 9	ns
IGF-I/IGFBP-1	1.458 $\pm$ 0.12	1.770 $\pm$ 0.30	<i>p</i> < 0.05

In normally cycling women, no variations were found in IGF-I or IGFBP-1 across the menstrual cycle, suggesting that the IGF system in the circulation may not normally play a major role for ovarian reproductive processes in humans (22). However, in ovarian hyperstimulation, both IGF-I and IGFBP-1 have been shown to vary in response to exogenous FSH (24, 38).

The normally circulating levels of IGFBP-1 are derived mainly from the liver, but in ovarian hyperstimulation there may be a contribution to circulating IGFBP-1 from hyperstimulated preovulatory follicles (48–50). The current finding of approximately tenfold higher levels of IGFBP-1 in follicular fluid as compared to levels in serum may support this idea. In the follicles, IGFBP-1 mRNA is present in granulosa cells of the dominant follicles (25,51) and has been reported to be increased around luteinization. The relatively higher levels of IGFBP-1 in the nonpregnant group may therefore, perhaps to some extent, reflect premature luteinization, a factor that is known to be associated with poor outcome in IVF (52).

However, the relation between the hormones involved in luteinization, i.e. LH, P4, and IGFBP-1, appears to be complex. The possibility of using increased serum levels of P4 at the day of hCG in IVF cycles to detect premature luteinization and to predict pregnancy has been examined by several investigators, and results of most studies show that P4 levels are not predictive of pregnancy (53–56). In our study, P4 levels in serum on the day of OPU were correlated to oocyte number and to E2 levels, but not to IGFBP-1 levels or to pregnancy.

In conclusion, we suggest that inhibin B may be useful as an early marker for ovarian reserve in the clinical management of controlled ovarian hyperstimulation. We also find that the ratio of IGF-I/IGFBP1 in serum before OPU and in follicular fluid at OPU may be used as an index of oocyte quality, and suggest further extended studies to validate this finding.

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