Vascular endothelial growth factor and inhibin A in follicular fluid of infertile patients who underwent in vitro fertilization with a gonadotropin-releasing hormone antagonist

João Sabino Cunha-Filho, Ph.D., Nadiane Lemos, M.D., Nina Stein, M.D., Andréia Laranjeira, M.D., and Eduardo Pandolfi Passos, Ph.D.

Human Reproduction Unit, Department of Obstetrics and Gynecology, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Objective: To investigate the role of a gonadotropin-releasing hormone (GnRH) antagonist, minimal stimulation protocol, in the follicular fluid by measuring vascular endothelial growth factor (VEGF) and inhibin A.
Design: A cross-sectional prospective study.
Setting: Academic hospital.
Patient(s): Seventy infertile patients submitted to in vitro fertilization (IVF).
Intervention(s): Patients were divided into two groups: group 1 (study) included 30 infertile patients subjected to IVF with a GnRH antagonist (minimal stimulation protocol); group 2 (control) included 40 infertile women who underwent natural-cycle IVF.
Main Outcome Measure(s): Follicular fluid VEGF and inhibin A measurements.
Result(s): The groups were comparable in terms of age, body mass index (BMI), and infertility characteristics. Moreover, follicular fluid VEGF and inhibin A concentrations (medians) were, respectively, 776 pg/ml (95% confidence interval [CI]: 775–1483) and 3,115 pg/mL (95% CI: 1,349–2,502) for group 1; 1,187.50 pg/mL (95% CI: 1,020–1,560) and 3,123.00 pg/mL (95% CI: 1,888–2,735) for group 2 ($P>.05$).
Conclusion(s): We demonstrated that GnRH antagonist administration in infertile patients undergoing IVF did not alter the follicular fluid content of VEGF and inhibin A, and, probably, maturation and quality of oocytes as well. These results demonstrated the usefulness and safety of this drug on controlled ovarian stimulation (COS) protocols. (Fertil Steril® 2005;83:902–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Inhibin A, VEGF, minimal stimulation, GnRH antagonist

The use of GnRH antagonists in human reproduction introduced a new perspective in terms of controlled ovarian stimulation (COS) and the possibility of alternatives to COS protocols.

In COS, the purpose of GnRH antagonists is to prevent or even block premature luteinizing hormone (LH) secretion. Several randomized trials compared cycles in which GnRH agonists or antagonists were administered, and some demonstrated a low number of retrieved oocytes in cycles with antagonists, despite similar pregnancy rates (1–6).

Most likely, the decreased number of oocytes obtained in cycles stimulated with antagonists is due to a different hormonal status and consequently, follicular cohort. Indeed, GnRH antagonists did not inhibit intercycle follicle-stimulating hormone (FSH) secretion, and follicular growth occurred in a completely different hormonal milieu from that of cycles using GnRH agonists (7).

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Reprint requests: Professor J.S. Cunha-Filho, Rua Ramiro Barcellos, 2350-11 andar, Serviço de Ginecologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RGS, Brazil. CEP 90035-003 (FAX: +55-51-21018148; E-mail: sabino@via-rs.net).

The minimal stimulation protocol was designed to achieve adequate reproductive results, but using a simpler and "friendlier" COS (8). This protocol utilizes a GnRH antagonist associated with a gonadotropin (recombinant FSH or human menopausal gonadotropin [hMG]) and induces a monofollicular development, reducing the costs and risks of a multiple gestation.

Vascular endothelial growth factor (VEGF) is produced by granulosa and theca cells in response to FSH, LH, and human chorionic gonadotropic (hCG). Primarily, it stimulates the mitogenic properties of endothelial cells and provokes angiogenesis, transforming the poorly vascularized preovulatory follicle into the well-vascularized corpus luteum (9, 10).

Some authors associate VEGF follicular fluid content with progesterone secretion, embryo maturation, number of administered gonadotropins, and follicular hypoxia (11, 12). Furthermore, others have reported that an increased follicular fluid VEGF concentration is also found in older patients and in poor responders, which is most likely a compensatory mechanism caused primarily by a hypoxic follicular environment (13–16).

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However, this statement is disputed by some investigators who have found that follicles with increased VEGF content had a higher dissolved oxygen concentration (17). Women who did not conceive after in vitro fertilization (IVF) or gamete intrafallopian transfer (GIFT) had a higher follicular fluid VEGF concentration than women who achieved a clinical pregnancy (16). This finding could be confirmed by studying the embryo morphology after COS (12): VEGF had a negative correlation with embryo morphology.

Inhibin A is a heterodimer composed of an α-subunit and one or two β_A-subunits ($\alpha\beta_A$). It is secreted throughout the menstrual cycle, and is responsible for inhibition of FSH, mainly during the luteal phase. Inhibin A secretion is regulated by LH and is associated with paracrine/autocrine action on oocyte maturation. Moreover, it is related to follicular development and size, serving as a marker of follicular maturation after IVF cycles (18–20).

Some authors discuss the effect of GnRH antagonists on oocyte and embryo quality (21); however, it was demonstrated an acceptable pregnancy rate even after embryo thawing in patients stimulated with a GnRH antagonist (22). Therefore, the effect of this drug on follicle development and, consequently, on oocyte competence was not properly assessed. The rationale of our study is to investigate the effect of a GnRH antagonist on oocyte quality and competence, considering that inhibin A and VEGF are both markers of oocyte maturity and development.

Thus, the aim of this study is to determine the follicular fluid concentration and the role of VEGF and inhibin A in infertile patients undergoing IVF using a GnRH antagonist (minimal stimulation) protocol.

MATERIALS AND METHODS

Design

A cross-sectional prospective study was performed, composed of 70 infertile patients subjected to IVF between January 2002 and October 2002.

Patients

The VEGF and inhibin A concentrations were assessed in the follicular fluid of 70 infertile patients who were subjected to IVF, which was the chosen treatment for infertility at the Human Reproduction Unit, Hospital de Clínicas de Porto Alegre, Brazil, between January 2002 and October 2002. Patients were informed about the procedures and signed an informed consent. The research project was approved by the Ethics Committee and registered at the Graduate Research Group of the hospital.

Patients were divided into two groups: group 1 (study) patients were subjected to an IVF protocol using minimal stimulation (n = 30); group 2 (control) patients underwent IVF using natural-IVF cycle (n = 40).

The following inclusion criteria were established: [1] patients with no previous endocrine disorders; [2] patients younger than 36 years; [3] patients with serum FSH levels below 10 IU/mL in the early follicular phase (day 3 of the menstrual cycle); and [4] BMI below 27 kg/m^2.

In Vitro Fertilization

On the third day of the first menstrual cycle after the beginning of the study protocol, we performed an ultrasound exam to exclude ovarian cysts. Briefly, group I patients received GnRH antagonist (0.25 mg) plus recombinant FSH (150 IU) daily when the dominant follicle reached 14 mm in diameter; hCG (5,000 IU) was administered when the dominant follicle reached 16 mm (8).

Alternatively, group 2 patients only received hCG (5,000 IU) when the dominant follicle had reached 16 mm in diameter. After the identification and separation of the oocyte, the follicular fluid was centrifuged at 2,500 rpm, so as to separate blood cells and other cell debris, and then frozen at –20°C for later assay. Wash buffer or contaminant was not used in our samples.

Measurements

Follicular fluid analysis was performed at the radioimmunoassay laboratory of Hospital de Clínicas de Porto

TABLE 1

Clinical and IVF characteristics (median and 95% CI).

	Group 1 (n = 30)	Group 2 (n = 40)	*P* value
Age (y) (range)	35.50 (32–36)	34.00 (33–35.50)	.533
BMI (kg/m^2) (range)	22.80 (21.80–25.30)	21.90 (21.50–23.20)	.267
Infertility	Primary 76% Secondary 24%	Primary 80% Secondary 20%	.771
Embryo transfer (%)	82.75	70.00	.268
Pregnancy rate (β-hCG)	13.30	7.50	.443

Note: IVF = in vitro fertilization; CI = confidence interval; BMI = body mass index; hCG = human chorionic gonadotropin.

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TABLE 2

Etiology of infertility.

	Group 1 (n = 30)	Group 2 (n = 40)
Endometriosis (%)	17.2	30.0
Tubal factor (%)	38.0	42.5
Male (%)	20.7	12.5
Unknown (%)	24.1	15.0

Note: $P=.449$ (Fisher's exact test).

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Alegre, with specific kits that did not cross-react with other analytes.

Inhibin A was measured by means of an enzyme-linked immunoabsorbent assay (ELISA) (DSL, Webster, Texas) with a minimal detection of 1 pg/mL and an intra- and interassay variation of 6.2% and 7.8%, respectively. In addition, VEGF was detected with the Sandwich ELISA-specific kit (Chemicon International Inc., Temecula, CA), with the detection limit set at 20 pg/mL, and intra- and an interassay variation of 8.9% and 11.1%, respectively.

Statistical Analysis

Because the variables showed a non-Gaussian distribution, the Mann–Whitney *U*-test was used. Otherwise, categorical data were analyzed using Fisher's exact test. Statistical significance was established when $P<.05$.

Sample size was calculated (power of 80%) to compare differences regarding VEGF and inhibin A levels in the follicular fluid.

Moreover, to investigate the effect of pregnancy on follicular fluid VEGF and inhibin A concentration, we divided groups 1 and 2 into two subgroups (pregnant and not pregnant patients).

RESULTS

In all patients, ovarian monofollicular development occurred as expected. Clinical characteristics and data regarding the analysis of ovulation induction from the two groups are presented in Table 1. Age and BMI were similar between the two groups ($P>.05$).

No differences were found regarding infertility characteristics (primary or secondary), embryo transfer, pregnancy rate, or oocyte/embryo quality (data not shown). Table 2 lists the etiology of the patients' infertility ($P=.449$).

Moreover, follicular fluid VEGF and inhibin A concentrations (medians) were, respectively, 776 pg/mL (95% confidence interval [CI]: 775–1,483) and 3,115 pg/mL (95% CI: 1,349–2,502) for group 1; 1,187.50 pg/mL (95% CI: 1,020–1,560) and 3,123 pg/mL (95% CI: 1,888–2,735) for group 2 ($P>.05$) (Fig. 1 and Fig. 2).

In addition, follicular fluid VEGF and inhibin A concentrations were not different comparing pregnant vs. nonpregnant patients (Fig. 3A and Fig. 3B, respectively).

DISCUSSION

We demonstrated that GnRH antagonist administration during controlled ovarian stimulation, using a minimal stimulation protocol, did not affect the follicular production of inhibin A and VEGF. In addition, comparing pregnant vs. nonpregnant patients, they were not statistically different, which increases the scientific importance of our results.

The role and importance of these peptides were evaluated by several investigators and are associated with luteal phase development, oocyte competence, and oxygenation and ovarian hyperstimulation syndrome (9, 18).

Several other studies (10–13, 15, 16) suggested that follicular fluid VEGF was linked to reproductive status or prognosis by several mechanisms:

1. Negative correlation with embryo morphology and follicular hypoxia

FIGURE 1

Follicular fluid VEGF concentration (pg/mL). The box represents the interquartile range, which contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Mann–Whitney *U*-test, $P=.375$.

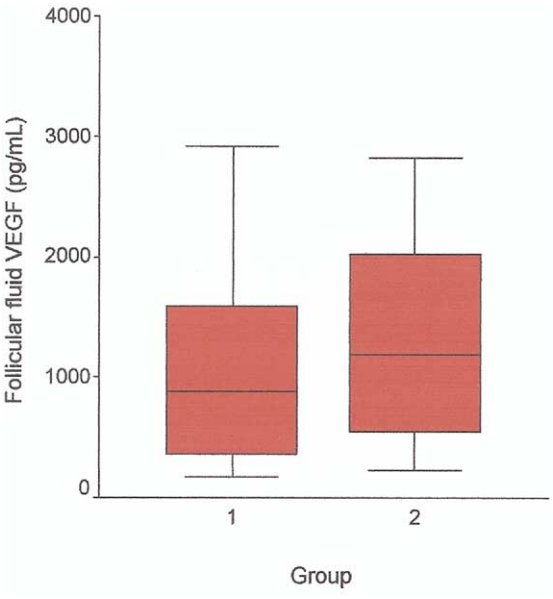

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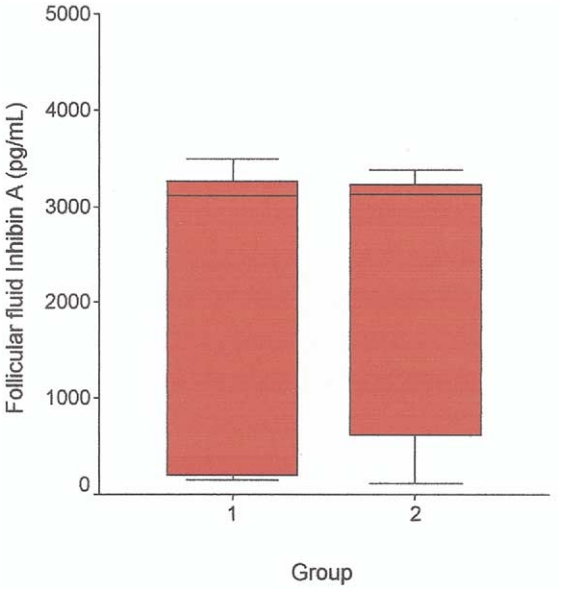
FIGURE 2

Follicular fluid inhibin A concentration (pg/mL). The box represents the interquartile range, which contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Mann–Whitney *U*-test, *P*=.765.



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2. Modulation of progesterone secretion (corpus luteum development)
3. Quantity of gonadotropin administered
4. Follicular fluid VEGF is high in older women, poor responders, and in association with low pregnancy rates, which is most likely due to a hypoxic intraovarian environment.

Some authors (23) demonstrated that follicular fluid VEGF concentration was enhanced in poor responders — most likely a compensatory mechanism to increase angiogenesis and to stimulate better oocyte maturation. Furthermore, follicular fluid VEGF in older women was also heightened, a fact that may be related to abnormalities of the meiotic spindle (14).

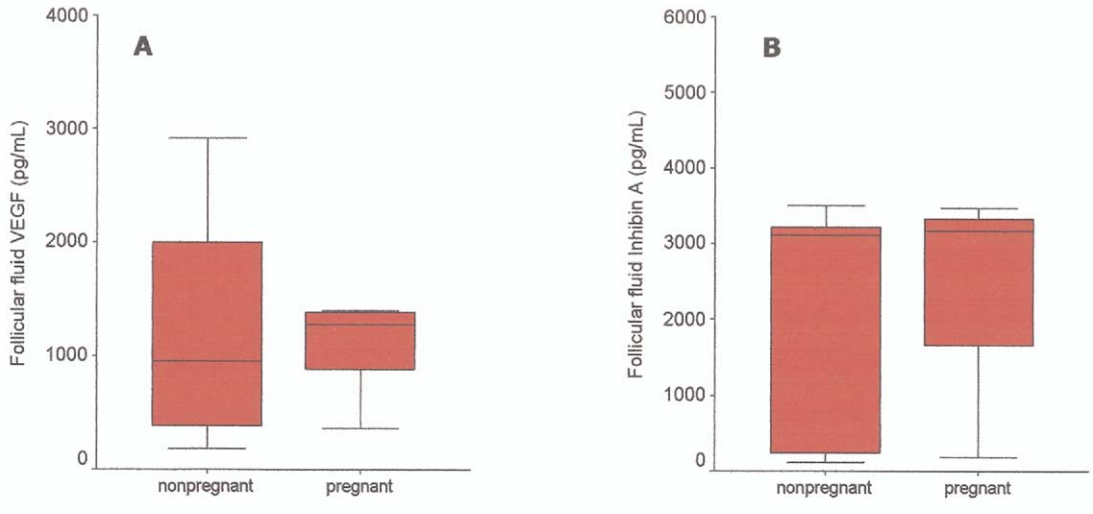
However, others have demonstrated that VEGF in follicular fluid was increased in well-vascularized follicles (17). The differences regarding VEGF results may be due to the fact that Van Blerkom et al. (17) included some severely hypoxic follicles (i.e., <1.5% dissolved oxygen) in their analysis.

In fact, VEGF had a negative correlation with embryo morphology and IVF outcome. Patients who became pregnant after IVF had a lower follicular VEGF fluid concentration than those women who failed after IVF (15).

Alternatively, inhibin A was not correlated with age (14). Nevertheless, this peptide was clearly linked to oocyte maturation (18, 24), had a paracrine/autocrine effect on follicu-

FIGURE 3

Comparison of (**A**) follicular fluid VEGF and (**B**) inhibin A concentrations (pg/mL) in pregnant and nonpregnant patients. The box represents the interquartile range, which contains the 50% of values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Mann–Whitney *U* test, *P*=.648 and .364, respectively.



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lar development (20), and was associated with ovarian stimulation and with pregnancy rates (25).

Inhibin A may also reflect the integrity of follicular development and capacity, perhaps serving as a marker of oocyte maturity (19), although not linked to oocyte fertilization capacity.

The GnRH antagonists are a new class of drugs for the treatment of infertility and have been compared with GnRH agonists (long protocol) in terms of reproductive outcomes. Although pregnancy rates are similar, the number of retrieved oocytes is lower in GnRH antagonist cycles. This fact was sufficient to initiate inquiries regarding the effect of GnRH antagonists on oocyte and embryo quality.

Differences in oocyte or embryo quality and in pregnancy or implantation rates were not demonstrated (2–5). However, these findings are disputed by other authors (26), who demonstrated in a meta-analysis that stimulation with GnRH antagonists displayed decreased implantation and pregnancy rates.

Recently, in a well-designed study, another group of investigators concluded that a small decrease in pregnancy and implantation rates occurs in GnRH antagonist/IVF donor cycles, which could be due to oocyte or embryo causes (27).

In fact, we analyze oocyte or embryo quality according to morphologic aspects and characteristics. Nevertheless, this method is subjective and not sufficiently accurate to detect small differences between the groups. Therefore, VEGF and inhibin A are two important peptides related to several reproductive mechanisms and IVF outcomes, which could be measured to investigate GnRH antagonist safety.

In this research, we analyzed the effect of a GnRH antagonist administration (minimal stimulation protocol) on VEGF and inhibin A concentrations in follicular fluid and demonstrated that this drug did not affect these important peptides.

More studies are needed to evaluate the effect of this drug on the molecular basis of oocyte and embryo development, however, mainly in controlled ovarian hyperstimulation cycles.

We focused and designed our research to study only monofollicular cycles (cases and controls), excluding some possible bias (e.g., number of follicles, discrepancy in follicle diameter/maturity, and gonadotrophin administration) when we compare hyperstimulated cycles. However, we must reinforce that it is very important to design similar studies that compare agonist vs. antagonist cycles.

The fact that the inhibin A and VEGF secreting capacity of granulosa cells is not altered after GnRH antagonist administration is also important in physiologic follicular development, oocyte competence, and luteal phase adequacy. It is possible that this drug may not affect follicular development and oocyte competence, as confirmed by the fertilization rate described in several randomized trials.

Moreover, inhibin A and VEGF could be associated with corpus luteum development and competence. Therefore, in cycles using GnRH antagonists, the luteal phase may not be dysfunctional, and the need for luteal phase supplementation may be questionable.

In conclusion, the administration of a GnRH antagonist did not alter the follicular fluid concentration of VEGF and inhibin A and, most likely, ovarian-follicular angiogenesis and oocyte maturity. This drug offers new perspectives and provides alternatives regarding ovarian stimulation protocols, which could increase reproductive probability while minimizing risks and costs.

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