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ASSISTED REPRODUCTION

Luteal phase oestradiol administration in ovarian stimulation cycles with GnRH antagonist is comparable to the GnRH agonist (long) protocol

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Abstract

Purpose: The purpose of the study was to compare the effectiveness of GnRH antagonist with luteal phase estradiol administration to GnRH agonist cycles, long protocol.

Methods: 55 IVF-ICSI patients received oestradiol in the luteal phase of the cycle, before a cycle with GnRH antagonist. Fifty-five patients submitted to IVF-ICSI with the use of agonist were allocated, age matched, as a control group (historical control). The primary outcome was the number of retrieved oocytes.

Results: Patients were similar in terms of clinical characteristics. No differences were found in the number of oocytes retrieved (study group, 8.1±4.7; control group, 7.4±4.5) or in oocyte quality.

Conclusions: We clearly demonstrated that the effectiveness of GnRH antagonist when combined with luteal phase estradiol is comparable to GnRH agonist cycles.

Keywords GnRH antagonists · GnRH agonist · Follicular cohort · Homogenization · Luteal phase oestradiol

Capsule Oestradiol associated to GnRH antagonist may increase the rates of oocytes causing reproductive results to be comparable to the results with the use of agonists.

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Introduction

The standard therapy for induction of ovulation for assisted fertilization consists in the administration of GnRH agonists during the previous luteal phase with the purpose of avoiding a precocious luteinization. Recently we have had the possibility of inhibiting a precocious liberation of the LH by GnRH antagonist administration.

The GnRH antagonists cause direct and immediate hypophisary suppression, which is the opposite of the agonists that require prolonged administration to obtain the same effect. Therefore, the antagonists can be administered immediately before the expected LH peak, and only a few days of treatment are necessary. Most of the studies suggest GnRH antagonist administration beginning on the sixth day of ovarian stimulation, with FSH or hMG use, for around 4 to 5 days [1–3].

During controlled ovarian stimulation cycles (COS) associated with the use of antagonists, these showed themselves to be equally effective as the agonists in the prevention of precocious luteinization. However, it was recently demonstrated that in cycles with antagonist there is a lower number of oocytes retrieved and lower serum oestradiol levels. Though this profile may have a favorable effect on the frequency of the ovarian hyperstimulation syndrome, it is responsible for lower levels of clinical pregnancies when compared to the utilization of agonists [4].

The lower number of mature oocytes probably should be related to a heterogeneous antral follicular cohort [5]. The inter-cycle increase of FSH during the late luteal phase is one of the most important steps in follicular development and recruitment [6]. We know that the secretion of FSH can be inhibited by the administration of oestrogen during the

luteal phase, which reduces the size and increases the homogeneity of the selectable follicles on the third day. Theoretically, the administration of oestradiol could increase the number of mature oocytes in the GnRH antagonist cycles in an attempt to reach better clinical results [7].

The objective of the present study is to compare the effectiveness of GnRH antagonist with luteal phase estradiol administration to GnRH agonist cycles, long protocol.

Materials and methods

Design

A clinical trial study was performed during the period from January 2003 to December 2004, with historic controls (matched by age) of the period from January 2001 to December 2002.

Patients

Patients eligible for IVF/ICSI that came to the Ob/Gyn Service at Hospital de Clínicas de Porto Alegre. The criteria of inclusion were the following: women between the age of 18 and 39 with a body mass index from 18 to 27 kg/m^2 and weight between 50 and 90 kg, and with menstrual cycles with duration from 24 to 35 days. The exclusion criteria were the following: contraindication to the use of GnRH antagonist, polycystic ovaries syndrome, history of poor response to the FSH, previous oophorectomy, more than three attempts of IVF/ICSI, BMI>27 kg/m^2.

Ovulation induction

Included patients in the study group received oestradiol (4 mg po daily) from the twentieth day to the second day of the following cycle and the COS was performed according the GnRH antagonist flexible protocol. The control group was made up of patients submitted to IVF/ICSI using a long protocol of GnRH agonist (leuprolide acetate 1 mg/day after the 20th day of the menstrual cycle) during 2001–2002. The historical controls followed the same criteria of exclusion and pairwise comparisons were made by age. Both groups received recombinant FSH in an initial dose of 150 IUs after the third day.

Ultra-sonographic monitoring was carried out after 5 days from the beginning of the stimulation with recombinant FSH and with a daily frequency up to the administration of the hCG. When at least three follicles were larger than 18 mm, the patients received the last injection of antagonist for the study group, and hCG (10,000 IU IM) were administered to both groups. The transference of embryos was done 72 h after the retrieval of the oocytes. The support of luteal phase was realized with 600 mg/day of micronized progesterone.

Approval was received from the Research Ethical Committee from Hospital de Clínicas de Porto Alegre and all of the patients were included only after they were informed about the procedures and signed an informed consent.

Statistical analysis

The primary outcome of the study was to evaluate the difference in the number of mature oocytes between the two groups. Secondary outcomes were the following: rates of fertilization and implantation; number of embryos in the second day of culture; number of embryos transferred; gestation rates, ovarian hyperstimulation syndrome (OHSS) and associated complications, quantity of gonadotropin used, and the ovulation induction time. Gestation was defined as an intrauterine gestation with at least one fetus with a heartbeat detected by ultrasound after 6 weeks of the embryo transference.

The calculation of sample size was done previously to beginning the study, considering Pβ 80% and Pα 5%. According to previous studies and taking into consideration an average number of oocytes retrieved of 8, with a standard deviation of 5 and a difference between the groups of 3 oocytes, an *n* was calculated of 50 patients under study and 50 controls.

Category variables were compared with chi-square test. Continuous variables were compared with Student *t* or Mann–Withney test, depending on its characteristics. We considered the differences to be statistically significant when $P<0.05$.

Results

One hundred ten patients were included: 55 patients in the study group (GnRH antagonist) and 55 controls (GnRH agonist). The mean age (±SD) of the control group was 32.8±4.4 years and of the study group was 32.6±4.0 years, without statistical difference. There are also no differences as to the causes of infertility between the groups, as demonstrated on Table 1. The parity of the patients and the duration of the infertility also did not present differences between the groups.

Only patients with tubal cause or masculine indication were included, which made a total of 66 patients that were submitted to IVF (tubal cause) and 44 to ICSI (masculine cause), this difference was not significant between the groups ($P>0.05$). Laboratory and clinical IVF/ ICSI results

Table 1 Clinical characteristics (values are expressed as means and SD)

	Group 1, oestradiol+antagonist (n=55)	Group 2, agonist (n=55)	P value
Age (years)	32.8±4.4	32.6±4.0	0.784
Duration of infertility (years)	5.2±3.0	5.4±2.5	0.784
Main causes of infertility			
Masculine (ICSI)	27	37	0.081
Anatomic (IVF)	28	18	

were presented in Table 2. No differences were found in the number of oocytes retrieved. The oocyte quality was similar in both groups. The rates of fertilization, implantation, and gestation, and the number of embryos transferred did not present differences between the groups (P>0.05). The number of days of induction and the total dose of gonadotropins were also similar in both groups. Another secondary outcome evaluated was the incidence of severe OHSS that was also similar between the groups. Six women of the study group and four from the control group presented OHSS (P=0.742, exact test of Fisher), and no patients needed to be hospitalized.

Discussion

Our study demonstrated that there was no difference between the GnRH oestradiol-antagonist group and the GnRH agonist group as for the primary outcome: the number of mature oocytes. There were also no differences when comparing the other reproductive outcomes.

Clinical trials that compared cycles with agonists versus antagonists presented similar reproductive results, though there is a small decrease in the number of oocytes retrieved in cycles with antagonists [2, 3, 8–10].

A plausible and recognized explanation is the lowering of the inhibin A and oestradiol levels at the end of the luteal phase with a consequential increase of the FSH inter-cycle [6]. This increase of FSH does not occur with patients submitted to ovulation induction with GnRH agonists that, by the action of the medication itself, has a reduction of the FSH levels and consequently a homogenization of the follicular cohort [5].

However, women that used antagonist do not have their follicular cohort prepared and may present an unfavorable hormonal environment with discrepancies between the selectable follicles [11]. These discrepancies could be the issues responsible for the differences found in relation to the number of retrieved oocytes.

Fanchin conducted a study published in 2003 in an attempt to prove that the differences in size between the antral follicles were a result, at least partially, to the precocious and progressive exposure to the FSH. So a random clinical trail was carried out with the use of oestradiol in the luteal phase versus a control group without medication. The administration of oestradiol had already shown to be effective in reducing the secretion of endogenous FSH [12, 13]. The ecographic and hormonal results on the third day of the following cycle proved the authors' hypothesis, with a reduction in the follicular diameter and discrepancies. These results demonstrate that the use of oestradiol in the luteal phase before COS with antagonist can be an instrument for the synchronization of the follicular growth [11].

In order to test the hypothesis of homogenization of the follicular cohort before the ovulation induction with antagonist, a clinical trial with 90 patients that were candidates for IVF was carried out by the same group of investigators [11]. Forty-seven patients received 4 mg of oestradiol beginning on the twentieth day of the previous cycle up to the second day of the cycle, and 43 patients served as controls. Follicles with a smaller average diameter

Table 2 Laboratory and clinical IVF/ ICSI results (values are expressed as means and SD)

	Group 1, oestradiol+antagonist (n=55)	Group 2, agonist (n=55)	P value
No of total oocytes retrieved	8.1±4.7	7.4±4.5	0.448
Fertilization rates	70%	72%	0.876
No of embryos transferred	2.7±1.2	2.7±1.2	0.917
FSH administered (IU)	1786.50±602.10	1620.83±346.50	0.093
Implantation rate	22%	21%	0.863
Clinical pregnancy	29%	27%	1.0

and with discrepancies of reduced size were found in the group of oestradiol as compared to the control group. The authors concluded that the administration of oestradiol in the previous luteal phase improves the follicular homogeneity, which is in accordance with our results.

Another aspect that is important to be is the use of a flexible or individualized protocol for COS with GnRH antagonist. In 2002 a study with 60 patients receiving cetrorelix under 3 different protocols was published: fixed multiple doses, individualized multiple doses, or individualized single dose. The number of oocytes retrieved was largest in the two groups of flexible protocol and the quantity of FSH used was lower in the protocol of flexible multiple dose. The results reinforce the hypothesis that the individual adjustment of GnRH antagonist medication optimizes the ovarian stimulation with a greater number of oocytes retrieved with lowering the need of FSH without increasing the need for monitoring [14].

Several clinical trials published in the beginning of this decade have similar results. They show that the use of antagonists reduces the treatment time with gonadotropins and the total dose used, as well as serum oestradiol levels [2, 3]. They also demonstrate the absence of a difference between antagonist and agonist in terms of reproductive outcomes [2, 3, 10].

However, with a systematic revision, the fixed protocol with antagonists demonstrated a lowering in the number of oocytes retrieved with a lower clinical gestation rate in relation to the cycles that used GnHR agonists and the idea of reducing the ovarian hyperstimulation syndrome in relation to the agonists was not confirmed [4]. Recently, we demonstrate there were no differences in inhibin A and VEGF in the follicular fluid of patients submitted to IVF with GnRH antagonist, comparing with natural cycles [15]. These results confirm the safety and effectiveness of the medication.

Some possible biases (measurement and confusion) of our study were controlled during the recruitment, measurement and statistical analysis as described below: (1) our primary outcome was the number of mature oocytes that is an unequivocal laboratory endpoint, (2) all patients were matched by age that is the main prognostic factor associated with almost all reproductive outcomes. Moreover, (3) we selected only patients who strictly matched the inclusion criteria.

We clearly demonstrated that, comparing GnRH antagonists to the long (agonist) protocol for COS is absolutely essential the pretreatment, during the luteal phase, with oestradiol is those patients submitted to COS using GnRH antagonist.

In conclusion, our study had enough proof to recommend the utilization of oestradiol in the luteal phase that precedes the COS with GnRH antagonists in order to make this protocol to COS comparable in efficacy to a GnRH agonist cycle. However, we thought it would be pertinent carry out a prospective random clinical trial in order to confirm our findings and to compare the usefulness of estradiol-antagonist protocol versus only the antagonist protocol.

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