Mild IVF using GnRH agonist long protocol is possible: Comparing stimulations with 100 IU vs. 150 IU recombinant FSH as starting dose

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Abstract

Purpose To test the possibility of stimulating ovaries with a mild IVF approach using a GnRH agonist long protocol.

Methods Retrospective study of 142 first IVF cycles of women aged 30 to 35 who had undergone stimulation with 100 IU or 150 IU of rFSH.

Results The mean dose of rFSH used was smaller in the low dose compared with the high dose group (999 vs. 1343 IU; $p < 0.001$), obtaining comparable numbers of mature oocytes in both groups. Additionally, reducing the mean number of embryos transferred from 1.8 to 1.5 significantly decreased the twin rate in the low dose group while maintaining similar pregnancy rates in both groups.

Conclusions It is possible to develop mild IVF using GnRH agonist long protocol in women with good prognosis. A reduced amount of rFSH and fewer transferred embryos allow for lower costs and risks of IVF without compromising the pregnancy rate.

Keywords GnRH agonist · IVF · Long protocol · Mild ovarian stimulation · rFSH

Introduction

Many authors are attempting nowadays to stimulate the ovaries with a mild approach in order to reduce patient

distress and the cost of in vitro fertilisation (IVF) cycles [1]. This new approach to ovarian stimulation has been found to render other benefits such as the probability of generating better embryos, a more receptive endometrium or a less overstimulated uterus.

Ovulation induction might induce oocyte abnormalities. Some of these abnormalities can be morphological [2, 3] or chromosomal [4]. Preliminary observations suggest that aneuploidy in embryos may not only be affected by maternal age [5], but also by the ovarian stimulation regimens employed in IVF [6]. A large prospective study used preimplantation genetic screening to analyse the effects of mild versus conventional stimulation on chromosome segregation behaviour during meiosis [7]. A mild protocol was defined as the use of 150 IU recombinant follicle stimulating hormone (rFSH) with gonadotrophin-releasing hormone (GnRH) antagonist co-treatment in short protocol; and a conventional stimulation was the use of 225 IU rFSH with GnRH agonist co-treatment in long protocol. They found that while the number of oocytes obtained with conventional IVF was higher than with mild IVF, the proportion of embryos with normal morphology was higher after mild stimulation and the proportion of chromosomally abnormal embryos and mosaic embryos per patient was significantly lower following mild stimulation. They concluded that ovarian stimulation strategies should avoid maximizing oocyte yield but concentrate on generating chromosomally normal embryos through reduced interference with ovarian physiology [7].

Ovarian stimulation has also been linked to adverse effects on endometrial receptivity, possibly due to advanced endometrial maturation and dysfunctional progesterone receptor activity [8–11]. Ovulation induction cycles with elevated oestrogen concentrations, compared to unstimulated cycles, are characterized by higher progesterone

Capsule Young women with good prognosis respond to 100 IU rFSH in a similar manner to 150 IU rFSH allowing for a lower cost of IVF.

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concentrations at the time of oocyte collection, and advanced histological dating with reduced pinopode formation at the time of embryo implantation [12]. This maturation of the endometrium may lead to embryo-endometrial asynchrony and reduced implantation rates in IVF cycles [13]. A more recent work also suggests that the gene expression pattern of the endometrium shows some differences when comparing natural cycles to stimulated cycles [14].

Ovarian stimulation may also negatively affect the implantation of embryos by stimulating more prominent junctional zone contractions in the myometrium [15, 16], which is presumably attributable to increased hormone concentrations. In contrast, contractions in mid-luteal phase in natural cycles, when implantation would be expected to begin, are hardly present at all [17].

Finally, there is a financial burden attached to ovarian stimulation, affecting either patients directly or national health systems, which should be taken into account. The use of high dosages of FSH or human menopausal gonadotrophin (HMG) is being increasingly questioned due to the increase in the cost of treatment, and because high dosages can cause significant side effects without evidence that they are of any benefit [1]. Some authors have already compared conventional ovarian stimulation (GnRH agonist long protocol and the transfer of two embryos) with mild IVF (GnRH antagonist co-treatment combined with single embryo transfer) from an economic perspective, finding the latter more advantageous per term live birth [18].

Over the last few years our reproduction unit has attempted a mild approach to perform the ovarian stimulation in young women (under 36), with normal ovarian function and a good prognosis, with the aim of improving the results of IVF while reducing the high cost associated with the treatment. We have analysed the data obtained from patients that had undergone the same GnRH agonist long protocol using 150 IU rFSH as starting dose for ovarian stimulation (between 2001 and 2003) versus 100 IU rFSH (from 2004 to 2007). The first objective of the study was to compare the results of the ovarian stimulation. The second objective was to compare implantation and pregnancy rates obtained. Clinical activity in the center and laboratory procedures were similar over the two periods of time except for a higher tendency to perform elective single embryo transfer and transfer in day 3 of embryo development in the latest period (details will be given).

Materials and methods

This is a retrospective study of 142 first IVF cycles (between 2001 and 2007) performed in the same number of women aged between 30 to 35, with normal regular menstrual cycles and body mass index (BMI) between 18

and 25. We excluded from the analysis women with polycystic ovarian syndrome, evidence of ovarian dysfunction, hyperprolactinaemia, ovarian failure, severe endometriosis, or elevated early follicular phase circulating FSH or oestradiol (E2) concentrations (FSH > 10 mIU/ml, E2 > 80 pg/ml).

Stimulation of the ovaries followed a long protocol using GnRH agonist (Nafarelin, Synarel®, 200 µg bid, High Wycombe, UK) which was started on the luteal phase (day 22 of the cycle). Pituitary down regulation was confirmed with transvaginal ultrasound and E2 levels < 50 pg/ml, and only then was the stimulation started. We analysed two groups: those starting with 100 IU of rFSH (Follitropin β, Puregon®; Organon Schering-Plough, Oss, The Netherlands) (*n*=79) and those starting with 150 IU of rFSH (Puregon®) (*n*=63). Stimulation was controlled by serial transvaginal ultrasound and serial serum E2 measurements starting on day 5 or 6 of stimulation, and doses were modified if needed from then onwards. HMG (HMG Lepori®, Farma Lepori, Barcelona, Spain) was administered to patients when E2 levels were considered to be low for the number of follicles observed by ultrasound (< 50 pg/ml per follicle ≥ 14 mm). Human chorionic gonadotrophin 10.000 IU (HCG Lepori®, Farma Lepori) was given when 1 to 3 follicles reached 18 mm. Oocyte pick-up was performed 35 h after HCG injection by transvaginal ultrasound-guided puncture of follicles. Conventional IVF or ICSI was performed and embryo transfer took place 2 or 3 days later. Embryos were scored according to morphological criteria (cell number, regularity of blastomeres, and fragmentation). Optimal morphology was defined as embryos with timely development (4 cells in day 2 or ≥ 6 cells in day 3), less than 20% fragmentation, and equal sized blastomeres. Luteal support was given in the form of micronized progesterone 200 mg/tid intravaginally (Utrogestan®, Seid, Barcelona, Spain or Progeffik®, Effik, Madrid, Spain) starting the day after oocyte retrieval.

Clinical pregnancies were those where a gestational sac was observed under ultrasound examination. The implantation rate was defined as the number of gestational sacs seen on transvaginal ultrasound divided by the total number of embryos transferred.

Outcome measures

Primary outcome measure was ovarian response, as assessed by the number of follicles ≥ 14 mm, oocytes retrieved, the number of mature oocytes, the dose of rFSH needed in each group, E2 on the day of HCG administration, days of stimulation and endometrial thickness. Secondary outcome measures were the fertilisation rate and embryological characteristics, implantation rate and pregnancy rates.

Statistical analysis

The data analysis was performed with SPSS version 13.0 for Windows.

Statistical analysis of continuous variables was performed with Student's *t*-test and the Levene's test was used to prove uniformity of variances. The calculated differences in means were expressed using 95% confidence intervals (CI). The χ^2 -test or Fisher's exact test was used to compare proportions between groups depending on the numbers involved. Statistical significance was assumed where $P < 0.05$. A power analysis is done for the primary outcome measures.

Results

Demographic and infertility characteristics for both groups are presented in Table 1. Despite analysing only cycles of women between 30 to 35 years, age was significantly different in the two groups ($p = 0.006$). BMI, basal FSH, basal oestradiol and the diagnosis of the patients were comparable in both groups. The most common diagnosis was male factor followed by unexplained infertility, tubal factor, mixed factor, mild endometriosis, uterine factor and coital factor. Fertility diagnoses are compared in both groups altogether and individually, using the chi-square test, and no statistical differences have been found.

The results of the ovarian stimulation are given in Table 2. Two cycles were cancelled in the 100 rFSH group (both due to poor response), and 6 cycles were cancelled in the 150 rFSH group (5 due to poor response and one due to risk of hyperstimulation) resulting in a similar percentage of women with oocyte retrievals in both groups. The number of days per stimulation, number of follicles ≥ 14 mm, levels

of oestradiol on the day of HCG and the percentage of patients who needed HMG were statistically similar in both groups. However, the mean dose of rFSH needed throughout the cycle was significantly lower in the 100 rFSH group than in the 150 rFSH group ($p < 0.001$). There was also a small (but significant) difference in endometrial thickness between the groups ($p = 0.048$). The number of recovered oocytes and mature oocytes were comparable in both groups. All women having follicles punctured had oocytes retrieved; however, one woman from the 150 rFSH group did not have mature oocytes.

The results of the insemination of the oocytes and embryological characteristics are given in Table 3. Conventional IVF, ICSI and combined insemination was used in the same percentage of cycles in both groups. The number of inseminated oocytes was lower in the 100 rFSH group compared to the 150 rFSH group ($p = 0.022$) and fertilisation rate was similar in both groups, so the total number of embryos obtained was significantly lower in the 100 rFSH than in the 150 rFSH ($p = 0.019$). The number of viable embryos was similar in both groups but the number of optimal embryos was slightly higher in the 150 rFSH group than in the 100 rFSH group ($p = 0.045$). Fertilisation failure was seen in five women in the 100 rFSH group while it didn't happen in the 150 rFSH group; however, this difference was not significant.

The results of the embryo transfers are given in Table 4. Fresh embryo transfer was cancelled in eight women of the 100 rFSH group and 1 of the 150 rFSH group. In the 100 rFSH group 7 embryo transfers were cancelled because of perceived risk of ovarian hyperstimulation, and one due of a diagnosed hydrosalpinx with hydorrhoea at the time of ovarian stimulation. In the 150 rFSH group one embryo transfer was cancelled due to lack of viable embryos.

Table 1 Demographic and infertility characteristics of IVF patients included in the two different treatment groups for ovarian stimulation

	100 rFSH group	150 rFSH group	P
Cycles	79	63	
Age ^a	33.1 $\pm$ 1.5	33.8 $\pm$ 1.3	0.006
BMI ^a	20.9 $\pm$ 2.9	21.6 $\pm$ 1.6	NS
Basal FSH ^a	6.7 $\pm$ 1.7	6.9 $\pm$ 1.6	NS
Basal oestradiol ^a	42.2 $\pm$ 16.9	42.7 $\pm$ 16.1	NS
Diagnosis: <i>n</i> (%)			
Mild endometriosis	3 (3.7%)	0	NS
Unexplained infertility	21 (26.5%)	23 (36.5%)	NS
Male factor	36 (45.5%)	25 (39.6%)	NS
Tubal factor	11 (13.9%)	5 (7.9%)	NS
Mixed factor	7 (8.8%)	7 (11.1%)	NS
Uterine factor	0	3 (4.7%)	NS
Coital factor	1 (1.2%)	0	NS

NS not significant

^a Values are mean $\pm$ SD

Table 2 Results of the ovarian stimulation of the two different treatment groups

	100 rFSH group	150 rFSH group	P	Power
No. Oocyte retrievals (%)	77 (97.4%)	57 (90.4%)	NS	
Days of stimulation ^a	8.9±1.4	8.8±1.3	NS	0.072
Follicles ≥14 mm ^a	11.6±4.6	11.2±5.2	NS	0.076
Endometrial thickness (mm) ^a	11±2.3	11.8±2.2	0.048	0.554
E2 day HCG (pg/ml) ^a	1381±685	1386±623	NS	0.05
Patients needing HMG	4 (5.1%)	4 (7%)	NS	
Dose of rFSH (IU) ^a	999±346	1343±299	<0.001	1
Oocytes retrieved ^a	9.7±4.1	10.2±5.3	NS	0.094
Mature oocytes ^a	7.3±3.4	8.1±4.7	NS	0.203
Cycles with no mature oocytes retrieved (%)	0	1 (1.7%)	NS	

NS not significant

^a Values are mean ± SD

Therefore, 64 women had a fresh embryo transfer in the 100 rFSH group compared with 55 in the 150 rFSH group (differences in transfer rate per cycle were not significant). Embryo transfers were performed in day 2 or 3 of embryo development in both groups; however, there was a significant increase in the number of transfers performed on day 3 ($p<0.001$) and a decrease on transfers on day 2 ($p<0.002$) in the 100 rFSH group compared with the 150 rFSH group. Pregnancy rates were not significantly different with transfers on day 2 or 3 either in total (63.3% vs. 55.1%) or within the groups (65.8% vs. 60.8% in the 100 rFSH group; and 61.2% vs 33.3% in the 150 rFSH group on days 2 and 3 respectively) and therefore results are given irrespective of the day of embryo transfer.

The number of embryos transferred was significantly lower in the 100 rFSH group compared with the 150 rFSH group ($p=0.006$). The implantation rate, pregnancy rate per cycle and per transfer were similar in both groups, but the twin pregnancy rate in the 100 rFSH group was significantly lower than in the 150 rFSH group ($p=0.012$). The

miscarriage rate was similar in both groups and there were no ectopic pregnancies. We also compared results from elective single embryo transfers (eSET) performed more frequently in the 100 rFSH group than in the 150 rFSH group ($p=0.002$) and obtained the same pregnancy rate.

Cancellations occurred for several reasons along the cycles (Table 5). The more important ones were the perceived risk of hyperstimulation, poor response and failed fertilisation. There were no reported cases of moderate or severe ovarian hyperstimulation after embryo transfer requiring hospitalisation in either group. However, the perceived risk of hyperstimulation provoked seven embryo transfers to be cancelled in the 100 rFSH group, and one cycle cancellation in the 150 rFSH group. Poor response was more frequent in the 150 rFSH group than in the 100 rFSH group. And finally, there were five cases of failed fertilisation in the 100 rFSH group while there were no such cases in the 150 rFSH group. There were a similar proportion of cycles cancelled in both groups and no significant differences in any of the problems resulting in cancellations.

Table 3 Results of the insemination and embryological characteristics in the two different treatment groups

	100 rFSH group	150 rFSH group	P
No. inseminated cycles	77	56	
Conventional IVF	6 (7.7%)	8 (14.2%)	NS
ICSI	31 (40.2%)	25 (44.6%)	NS
Combined IVF–ICSI	40 (51.9%)	23 (41%)	NS
Inseminated oocytes ^a	5.9±2.1	7±3.1	0.022
Fertilisation rate (%) ^a	64.3%	70.4%	NS
Total no. of embryos ^a	3.9±2.1	4.9±2.7	0.019
Viable embryos ^a	3.1±1.9	3.8±2.2	NS
Optimal embryos ^a	1.6±1.3	2.3±2	0.045
No. fertilisation failure	5 (6.4%)	0	NS

NS not significant

^a Values are mean ± SD

Table 4 Results from the embryo transfers in the two different treatment groups

	100 rFSH group	150 rFSH group	P
Cycles with embryos	72	56	
No. Transfers (%)	64 (88.8%)	55 (98.2%)	NS
Transfer on day 2	41 (64%)	49 (89%)	0.002
Transfer on day 3	23 (35.9%)	6 (10.9%)	0.001
Embryos transferred ^a	1.5±0.5	1.8±0.4	0.006
Pregnancies	41	32	NS
Implantation rate	48%	45%	NS
Single pregnancies (%)	29 (70.7%)	16 (50%)	NS
Twin pregnancies (%)	6 (14.6%)	13 (40.6%)	0.012
Miscarriages (%)	6 (14.6%)	3 (9.3%)	NS
Pregnancy rate/cycle	51.8%	50.7%	NS
Pregnancy rate/transfer	64%	58.1%	NS
Elective SET (%)	27 (42.1%)	9 (16.3%)	0.002
Pregnancies eSET (%)	15 (55.5%)	5 (55.5%)	NS

NS not significant

^a Values are mean ± SD

Discussion

A mild IVF cycle is defined (by the ISMAAR association) [19] as the method when FSH or HMG is administered at lower doses, and/or for a shorter duration in a GnRH antagonist co-treated cycle, or when oral compounds (anti-estrogens or aromatase inhibitors) are used either alone or in combination with gonadotrophins. The aim is to collect between 2 and 7 oocytes. Nargund *et al.* [19] stated that in theory, it might be possible to develop mild IVF protocols using GnRH agonist, but sustained that such data was unavailable. Our study seeks to fill that gap.

We are aware that the present study has two main problems. The first one is that it is a retrospective analysis and therefore it cannot guarantee that all clinical and laboratory decisions were applied in an identical way to the groups compared. However, the stimulation of the ovaries in the patients analysed was performed following the same protocol, using the same GnRH agonist and rFSH, and therefore, we believe that conclusions on the results of

the ovarian stimulation with different doses of rFSH can be properly compared. The second problem comes from the significant difference observed in the age of the two groups compared, despite including in the analysis only patients between 30 and 35 years of age. The difference between the mean values of the two groups was 0.7 years with a confidence interval between 1.2 and 0.21 years. We do not believe that this difference will have greatly influenced the clinical response of the ovaries to the stimulation since the difference between the groups is not higher than 1.2 years with a 95% confidence and other characteristics relevant to ovarian response, such as basal FSH, basal oestradiol and diagnosis of the patients in both groups, did not differ.

The analysis of the data was done retrospectively since it arises from a clinical decision taken at the time of a change in Spanish law in 2004 that forbade obtaining more than three embryos per patient per cycle. A lower starting dose of rFSH was then used to stimulate the ovaries in patients with good prognosis and normal ovarian function, expecting a lower recruitment of follicles. Individual response to ovarian

Table 5 Cycle cancellations

	100 rFSH group (n=79)	150 rFSH group (n=63)	P
Risk of OHSS	7 (8.8%)	1 (1.5%)	NS
Poor response	2 (2.5%)	5 (7.9%)	NS
Failed fertilisation	5 (6.3%)	0	NS
No mature oocytes	0	1 (1.5%)	NS
Hydorrhoea	1 (1.2%)	0	NS
No viable embryos	0	1 (1.5%)	NS
Total cancellations	15 (18.9%)	8 (12.6%)	NS

NS not significant

stimulation is highly variable and a need of an increase of rFSH during the stimulation in order to maintain the growth of the follicles was also expected. Surprisingly, patients recruited a similar number of follicles when starting with 100 or 150 rFSH, and it was possible to maintain the dose of rFSH in many patients over a similar number of days of stimulation in both groups, finally recovering a similar number of mature oocytes and obtaining similar levels of serum oestradiol on the day of HCG. It was important to notice that the main difference between patients starting with 100 versus 150 rFSH was that they used 344 IU less of rFSH ($p < 0.001$) decreasing the cost of the treatment.

Our results in the number of oocytes retrieved from the low dose group (9.7 oocytes retrieved with a total dose of 999 IU rFSH given over 8.9 days) are in the range of those obtained by other authors who used 100 IU Puregon® as starting dose in long agonist protocols, although they employed a higher dose over a longer period of stimulation. Devroey *et al.* [20] in a study with 51 patients, obtained a mean of 11.7 oocytes with a total dose of 1,807 IU of rFSH given over 13.3 days; and Moon *et al.* [21] in a study with 40 patients, obtained 8.4 oocytes with a total dose of 1,480 IU rFSH given over 10.8 days.

Other authors [22] have compared the effect of a starting dose of 100 IU vs. 200 IU Puregon® in patients between 18 and 39 years of age, and reached conclusions different from ours. They found that the 200 IU group used more rFSH than the 100 IU group (1,931 vs. 1,114 IU), but also had a significantly higher number of retrieved oocytes (10.6 vs. 6.2). They found no difference in the pregnancy rate per started cycle between the two starting doses, but concluded that 100 IU fixed dose was less efficacious than 200 IU due to the lower number of oocytes retrieved. Differences between this study and ours could be due to the age of the patients included or to the doses of rFSH compared; furthermore, the agonist used might also have an effect on the results. Out *et al.*'s study was multicentered. Variability in the results obtained in the 100 IU group between the centres was important and raised doubts as to the efficacy of the dose. The mean number of oocytes retrieved in the 100 IU group ranged from 1.6 to 7.5 among five centres, and the cancellation rate due to low response ranged from 3 to 79%. The dose and type of GnRH agonist used for the stimulation was not defined in the study and therefore was different among the centers (nafarelin, buserelin and leuprolide). Our study has the advantage of including younger patients and using a single type and dose of GnRH agonist, which may account for the higher number of oocytes retrieved and the low cancellation rate due to poor ovarian response. It is possible that the use of a low dose of nafarelin (200 µg bid) in our study (half of what is recommended by the manufacturer), might have a positive effect on the stimulation, lowering the amount of rFSH

needed to stimulate the ovaries. A similar effect was observed in a previous study, by our group [23], using leuprolide 0.1 ml/day instead of 0.2 ml/day for down-regulation. In a prospective study with 50 patients the use of leuprolide at a lower dose allowed a significant reduction of the number of ampoules of urinary FSH used (27 vs. 33 ampoules) to obtain a comparable number of oocytes in both groups, stimulating for the same number of days.

In our study, the number of oocytes inseminated in the 150 rFSH group was significantly higher than in the 100 rFSH group, despite having the same number of oocytes retrieved from both groups. This was due to the influence of the Spanish law during 2 years of our study (2004 to 2006) which forbade the insemination of all oocytes; although additionally, the number of inseminated oocytes may vary depending on patients' wishes. The insemination of a lower number of oocytes, with similar fertilisation rates, led to a lower total number of embryos and optimal embryos in the 100 rFSH group compared with the 150 rFSH group.

Due to a progressing policy of reducing the number of embryos transferred, most patients in the 150 IU rFSH group were recommended the transfer of two embryos, while in the 100 rFSH group more patients were encouraged to have an elective single embryo transfer (eSET) if there was at least one embryo of optimal morphology. We therefore transferred significantly fewer embryos in the 100 rFSH group than in the 150 rFSH group and obtained similar implantation rates, pregnancy rates per cycle and per transfer which might suggest a tendency to obtain better results with the lower dose of rFSH. However, when considering only the results in elective single embryo transfers, the pregnancy rate was the same in both groups suggesting a similar quality of the embryos transferred and preparation of the endometrium (despite having a thicker endometrium in the 150 rFSH group compared with the 100 rFSH group). Our results are similar to those found in other studies that report better treatment outcomes with lower doses of gonadotrophins. Stadmauer *et al.* [24] showed in a retrospective analysis of 264 cycles with embryo transfer, after agonist down-regulation, that both a higher average daily dose and a higher total dose per cycle of gonadotrophins were associated with lower clinical pregnancy rates. In another study Ben-Rafael *et al.*, [25] found fewer atretic oocytes ($p < 0.05$) and a higher pregnancy rate (22.8 versus 10.5%, not significant) in patients receiving two ampoules of HMG compared to those receiving three ampoules. Some studies comparing the use of two different doses of rFSH in antagonist cycles (150 vs 200 rFSH or 150 vs. 225 rFSH) found that the number of oocytes retrieved was higher using a higher dose of rFSH ($p < 0.05$), but the proportion of high quality embryos and implantation rate were higher with the lower dose of rFSH (not significant) [26, 27]. Out *et al.* [22, 28] comparing starting doses of 100 IU vs 200 IU rFSH or

150 IU vs. 250 IU rFSH in long agonist protocols also found higher pregnancy rates and implantation rates with the low-dose regimens, although differences were not statistically significant. Most of the studies mentioned, like ours, were not set up primarily to investigate pregnancy rates and their statistical power was too low to detect clinically meaningful differences for that parameter, so no firm conclusions can be drawn from this.

The reduction of embryos transferred allowed us to reduce the rate of twins in the low dose group. Already Heijnen *et al.* [29] compared results obtained with a mild treatment (ovarian stimulation with GnRH antagonist co-treatment combined with single embryo transfer) with a standard treatment (stimulation with a GnRH agonist long protocol and transfer of two embryos) with results that showed similar cumulative pregnancy rates with the two approaches, lower multiple pregnancy and lower costs with the mild approach, and no differences in anxiety, depression, physical discomfort or sleep quality of the mother. Elective single embryo transfer with subsequent cryopreservation of other viable embryos could further increase the benefits from the mild strategy. Moreover, mild approaches may reduce patient dropout and thereby improve cumulative birth rates as predicted by Edwards *et al.* [30].

The percentage of cycles that were cancelled was similar between the two groups but happened at different times of the cycle. It was surprising to find that poor response (≤ 3 follicles ≥ 14 mm) was the main reason for cancelling cycles in the 150 rFSH group, while the perceived risk of ovarian hyperstimulation ($\geq 3,000$ pg/ml or ≥ 20 follicles ≥ 14 mm on the day of HCG) was more frequent in the 100 rFSH group and entailed the cancellation of seven fresh embryo transfers. We believe that these results are difficult to explain in a retrospective analysis, when clinical protocols are followed but influenced by patients' decisions. On the other hand, there is no explanation for the increased (although not significantly) number of cycles with failed fertilisation in the 100 rFSH group. Neither diagnosis of the couples (male factor in two cases, 1 unexplained infertility, 1 coital factor and 1 mixed factor), or the type of insemination (3 ICSI and 2 conventional IVF) can account for the unexpected results. It is to be mentioned, however, that there were a low number of oocytes inseminated (1, 2, 3, 4 and 5 oocytes).

In conclusion, our study demonstrates that it is possible to develop mild IVF using the long GnRH agonist protocol. Young women with good prognosis respond to a low dose of rFSH (100 IU) in a similar manner to higher doses of rFSH (150 IU). Results in the number of oocytes, embryos and pregnancy rates are similar, and using a significantly lower amount of rFSH allows IVF to be performed at a lower cost. Additionally, it is concluded that a reduction in the number of embryos transferred in young women decreases the twin rate without compromising the pregnancy rate.

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