

Article

GnRH antagonists in ovarian stimulation for ICSI with oocyte restriction: a matched, controlled study



Ermanno Greco is the scientific director at the European Hospital Reproductive Medicine Unit in Rome, Italy. He graduated from the Medical School of the University of Rome in 1982. He became Researcher in Andrology at the University of Rome in 1985 and then Professor in Andrology there. Subsequently he has also been appointed Professor in Andrology at the University of Pisa. His current areas of interest include male infertility, azoospermia treatment, sperm DNA fragmentation, impotence and drug delivery devices.

Dr Ermanno Greco

E Greco^{1,3}, K Litwicka¹, S Ferrero¹, E Baroni¹, F Sapienza¹, L Rienzi¹, S Romano¹, MG Minasi¹, J Tesarik²

¹Assisted Reproduction Centre, European Hospital, Via Portuense 700, 00149 Rome, Italy; ²Mar&Gen, Molecular Assisted Reproduction and Genetics, Gracia 36, 18002 Granada, Spain

³Correspondence: Tel. +39 6 8842213, Fax: +39 6 8842286, e-mail: ergreco1@virgilio.it

Abstract

Italian legislation regarding reproductive medicine limits the number of embryos transferred per attempt to three. Thus, in order to achieve pregnancy, more IVF cycles may be required, generating a need for methods of ovarian stimulation with fewer side effects. The gonadotrophin-releasing hormone (GnRH) antagonists have several advantages in this respect, but there is a debate regarding a possible lower pregnancy rate from resulting cycles. This study evaluated the clinical applicability of GnRH antagonists for ovarian stimulation in young women undergoing intracytoplasmic sperm injection (ICSI) in which only three oocytes can be fertilized. The 200 women treated with GnRH antagonist had a significantly shorter stimulation and lower gonadotrophin consumption, oestradiol concentration, total and mature oocyte recovery as compared with 200 matched controls treated with GnRH agonist. No differences were found between the groups in the number of normal zygotes, total cleaved, transferred and high quality embryos, or in the clinical outcomes. Thus, the previously reported lower pregnancy rate in GnRH antagonist cycles may be related to the oocyte characteristics. Finally, under conditions of oocyte number restriction, the GnRH antagonist-based cycles may be proposed as an efficacious, safe and minimally invasive alternative to GnRH agonist in a standard long protocol.

Keywords: GnRH antagonist, ICSI, oocyte restriction, ovarian stimulation

Introduction

Since 10 March 2004, Italian reproductive medicine has been regulated by a new law that limits the number of embryos transferred per attempt to three, and prohibits their selection and cryopreservation, except for the situation in which the transfer of fresh embryos is impossible (Benagiano and Gianaroli, 2004). In practical terms, it means that the number of oocytes to be used in IVF programmes has to be limited to three.

One year after the application of oocyte restriction, a multicentre Italian study evaluated the impact of this new policy on clinical outcome, concluding that a strategy of fertilization of a maximum of three oocytes and the non-selective transfer of all obtained

embryos has a limited negative impact on the success rate of IVF cycles using fresh embryos, while the prohibition on freezing embryos seems to result in a more significant negative impact (Ragni *et al.*, 2005). However, these conclusions were made on the basis of analysis of a heterogeneous patient population, and no information was given with regard to the clinical results from different treatment protocols in specific subpopulations of women.

In such conditions of reproductive medical practice in Italy, in order to achieve pregnancy, it may be necessary to undergo comparatively more IVF/intracytoplasmic sperm injection (ICSI)

cycles, exposing some women, mainly those under 40 years, to an increased risk of treatment side-effects. Therefore, there is a tendency for Italian couples to follow 'procreative tourism' to other European countries in the conviction that different and sometimes less restrictive legislation may result in a quicker, easier and safer solution to their infertility problems (Cohen, 2006; Sauer, 2006). This situation requires particular attention to be paid to clinical outcomes in terms of risk/benefit and cost/effectiveness, and to the increasing demand by women for 'friendly' ovarian stimulation protocols.

The availability of GnRH antagonists for ovulation inhibition opened the way for the development of novel milder, simpler and safer ovarian stimulation. The clinical application of these methods demonstrated several advantages for women, including reduced duration of stimulation, lower total dose of gonadotrophins required for ovarian stimulation, no risk of cyst formation, no hormonal withdrawal symptoms and lower rate of ovarian hyperstimulation symptoms (Albano *et al.*, 2000; Born and Mannarets, 2000; Olivennes *et al.*, 2000; European and Middle East Orgalutran Study Group, 2001; Fluker *et al.*, 2001; Al-Inany *et al.*, 2006; Kolibianakis *et al.*, 2006). However, in the cycles utilizing GnRH antagonists, the number of oocytes retrieved and the pregnancy rate tended to be lower compared with the conventional long GnRH agonist protocol (Albano *et al.*, 2000; Born and Mannarets, 2000; Olivennes *et al.*, 2000; European and Middle East Orgalutran Study Group, 2001; Fluker *et al.*, 2001; Ludwig *et al.*, 2001; Al-Inany *et al.*, 2006; Kolibianakis *et al.*, 2006). In addition, the difference in the pregnancy rate became significant when data from individual studies were subjected to a meta-analysis (Al-Inany *et al.*, 2002, 2006; Aboulghar 2004). However, a recent systematic review and meta-analysis suggests that the probability of live birth is only slightly lower in cycles using GnRH antagonists for suppression of premature LH surge (Kolibianakis *et al.*, 2006).

The suggestion that GnRH antagonists are less efficacious than GnRH agonists in the long protocol raises a concern that their employment may aggravate the negative impact of the legislation on the clinical results of Italian IVF/ICSI programmes.

In addition, the question whether the presumptive adverse effect of GnRH antagonists on the pregnancy rate is mediated by their action on follicular development, by interference with corpus luteum function or by deterioration of the uterine environment, still remains open (Hernandez, 2000; Saadat *et al.*, 2004; Kolibianakis *et al.*, 2005; Tarlatzis *et al.*, 2006). Specific restriction policies imposed on IVF practice in Italy inadvertently offer a scientifically valuable model with which such a question can be addressed.

Given this background, the objective of this study was to assess the clinical applicability of GnRH antagonists in Italian ICSI programmes in the population of young women with normal ovarian reserve. The study also aimed to clarify possible causes of diminished clinical success in the cycles using GnRH antagonists.

So far as is known, this is the first matched and controlled study comparing the efficacy of GnRH antagonists and GnRH agonists in ovarian stimulation for ICSI under conditions of legally imposed oocyte restriction.

Materials and methods

This study involved 200 sequential ICSI attempts performed between December 2004 and December 2005 with the use of a GnRH antagonist protocol. Inclusion criteria were age <40 years, body mass index between 18 and 29 kg/m², regular (21–35 days) spontaneous menstrual cycles, presence of both ovaries, normal ovarian reserve as defined by FSH and oestradiol concentrations and ultrasound appearance of the ovaries (Smotrich *et al.*, 1995; Scheffer *et al.*, 1999), and neither uterine nor ovarian anomalies detected at ultrasound. None of the patients received an oral contraceptive on the menstrual cycle before ovarian stimulation. Exclusion criteria were history of a poor response in previous treatment cycles and azoospermia or other male pathology requiring testicular sperm retrieval.

For each of these cases, a matched control was selected from women undergoing GnRH agonist-based ovarian stimulation. Matching criteria were as follows: age of the patient (± 1 year), basal serum FSH concentration and rank of trial. In the event that several patients were found for these criteria, the one to be matched was randomly chosen. The matching was carried out blindly, without the knowledge of other relevant clinical data or outcome of the ICSI trial.

Patients could enter the study only once, and in order to be analysed should have received human chorionic gonadotrophin (HCG). The ovarian stimulation protocol and laboratory techniques for ICSI and embryo culture remained unchanged throughout the period of analysis. Informed consent was obtained from all patients participating in the study.

In the group of patients undergoing ovarian stimulation based on the use of GnRH agonist, pituitary down-regulation was achieved with the use of buserelin acetate (Suprefact; Aventis Pharma, Milan, Italy) 0.2 mg twice daily, started on day 21 of the menstrual cycle. After subsequent vaginal bleeding and pituitary down-regulation (oestradiol concentration <40 pg/ml and no ovarian cystic structures on ultrasound examination), ovarian stimulation was started with the use of recombinant human FSH (Puregon; Organon, Rome, Italy). The starting dose of FSH was chosen according to the patient's age and basal serum FSH concentration, and it was continuously adapted according to serum oestradiol concentrations and the dynamics of ovarian follicular growth as described previously (Tesarik *et al.*, 2001). Ovulation was induced with 10,000 IU of HCG (Gonasi; Amsa, Rome, Italy), when serum oestradiol exceeded 1000 pg/ml and at least three follicles had reached a mean diameter of 18 mm. Oocyte retrieval was performed 36 h after HCG administration under transvaginal ultrasound-guided puncture of the follicles.

The women undergoing ovarian stimulation using GnRH antagonist started the recombinant human FSH (Puregon) on day 2 of the cycle when serum oestradiol and progesterone concentrations were <80 pg/ml and <1.6 ng/ml respectively. Otherwise, FSH administration was delayed or the cycle was cancelled according to the published criteria (Kolibianakis *et al.*, 2004). The dose of FSH was determined as above. GnRH antagonist ganirelix (Orgalutran; Organon) was started on day 6 of FSH therapy and continued, at a daily dose of 0.25 mg, until at least three follicles reached 17 mm in diameter. At this time, ovulation was induced with 10,000 IU HCG (Gonasi), followed by transvaginal, ultrasound-guided oocyte retrieval 36 h later.

In both groups of patients, the luteal phase was supported with natural micronized progesterone (Prontogest; Amsa) administered i.m. in one daily dose of 50 mg. The treatment was started on the day of oocyte retrieval and continued until the day of the pregnancy test, performed 13 days after the embryo transfer. In the case of a positive test, this regimen was continued during the first pregnancy trimester. Clinical pregnancy was defined as the presence of a gestational sac with positive heartbeat.

Furthermore, on the day of the HCG test before undergoing blood sampling, all participants were asked to report adverse experiences during the treatment period and to express their opinion concerning the modality of ovarian stimulation. Overall patient satisfaction with the treatment regimen was classified as greatly satisfied, moderately satisfied, neither satisfied nor dissatisfied, or dissatisfied.

The three oocytes subjected to ICSI were selected from the whole metaphase II (MII) oocyte cohort according to the previously described criteria of oocyte and polar body morphology and meiotic spindle evaluation using the PolScope system (Rienzi et al., 2003). Fertilization was assessed 16–18 h after ICSI. On this occasion, zygotes were scored as good and poor morphology according to criteria based on the assessment of the number and distribution of nucleolar precursor bodies in the pronuclei (Tesarik and Greco, 1999). The two-pronucleated (2PN) zygotes were cultured further, under the same conditions, for an additional 2 days. At the time of medium change on days 2 and 3, embryos were assessed again with the previously described scoring criteria (Tesarik and Greco, 1999). Embryos were transferred 3 days after ICSI with the use of a K-JETS-7019-SIVF embryo-transfer set (Cook, Queensland, Australia).

Sperm-injected oocytes, zygotes and embryos were incubated at 37°C in G-FERT medium (Vitrolife, Goteborg, Sweden) equilibrated with 6% CO₂ in air.

Differences between groups were assessed by two-tailed chi-squared tests with Yates' correction or Fisher's exact test for categorical variables, and by the Mann–Whitney *U*-test for continuous variables. All analyses were performed using the Statistica 5.0 package (Statsoft Version 5.1, Hamburg, Germany).

Results

There was no difference in the distribution of patients with different causes of infertility between the GnRH antagonist group and the matched GnRH agonist group. Basic patient characteristics, including female age, male age, number of previous ICSI attempts, cycle length and baseline hormone concentrations were also comparable in both groups (**Table 1**).

In the GnRH agonist group, all women started the ovarian stimulation between days 2 and 4 of the menstrual cycle and no cycle delay or cancellation due to incomplete pituitary down-regulation was noted. In the GnRH antagonist subpopulation, gonadotrophin initiation was postponed for 1–2 days in 10 (5%) cases because of initially elevated progesterone concentrations (2.2 ± 0.4 ng/ml). The hormonal data obtained from both groups on the day of starting FSH, as well as LH concentration on day 1 of ganirelix administration, are presented in **Table 2**.

The ovarian stimulation characteristics differed significantly between both treatment groups, showing that GnRH antagonist-based cycles were of shorter duration ($P < 0.05$), required a lower total dose of FSH ($P < 0.01$) and resulted in lower peak serum oestradiol concentrations ($P < 0.01$) and fewer total and mature oocytes recovered (both $P < 0.05$) compared with cycles using GnRH agonist (**Table 2**).

In spite of the higher number of mature oocytes retrieved in the GnRH agonist group, the number of oocytes subjected to ICSI did not differ between the groups, in compliance with the legally imposed restriction of a maximum of three of oocytes used for attempted fertilization.

The number of normal (2PN) zygotes, cleaved and transferred embryos obtained from the GnRH antagonist regimen was comparable with those obtained from GnRH agonist protocol. Similarly, with comparable numbers of transfer procedures, neither the number of embryos transferred per cycle nor the number of embryos graded as good morphology differed between women included in the different ovarian stimulation protocols. No statistically significant difference was found in overall or clinical

Table 1. Demographic and clinical characteristics of patients included in the study.

Characteristic	GnRH antagonist protocol (n = 200)	GnRH agonist protocol (n = 200)
Female age (years)	33.0 \pm 2.2	32.6 \pm 2.6
Male age (years)	34.4 \pm 2.7	34.1 \pm 2.8
Aetiology n (%)		
Female factor	84 (42.0)	81 (40.5)
Male factor	77 (38.5)	74 (37.0)
Couple factor	27 (13.5)	30 (15.0)
Idiopathic factor	12 (6.0)	15 (7.5)
No. of previous attempts	1.2 \pm 0.3	1.1 \pm 0.3
Cycle length (days)	28.1 \pm 1.1	28.2 \pm 0.9
Baseline FSH (IU/l)	7.2 \pm 0.7	7.1 \pm 0.8
Baseline oestradiol (pg/ml)	28.0 \pm 13.0	5.0 \pm 10.0

Values are mean \pm SD, unless otherwise stated. GnRH = gonadotrophin-releasing hormone. There were no statistically significant differences between the two groups.

Table 2. Ovarian stimulation cycle characteristics for women treated with gonadotrophin-releasing hormone (GnRH) antagonist or agonist protocol.

<i>Cycle characteristic</i>	<i>GnRH antagonist protocol (n = 200)</i>	<i>GnRH agonist protocol (n = 200)</i>	<i>P-value</i>
Oestradiol at rFSH initiation (pg/ml)	32.5 ± 10.9	22.7 ± 8.9	–
Progesterone at rFSH initiation (ng/ml)	0.8 ± 0.2	–	–
LH at ganirelix initiation (IU/l)	3.0 ± 0.9	–	–
Stimulation duration (days)	11.1 ± 0.3	12.2 ± 0.4	<0.05
Total rFSH dose (IU) ^a	1633 ± 225	2212 ± 319	<0.01
Peak oestradiol concentration (pg/ml)	988 ± 129	1383 ± 197	<0.01
No. of oocytes retrieved ^a	6.8 ± 1.1	9.9 ± 1.6	<0.05
No. of MII oocytes retrieved ^a	4.4 ± 1.0	7.9 ± 1.3	<0.05

^aPer stimulated cycle.

Values are mean ± SD, unless otherwise stated; MII = metaphase II; rFSH = recombinant FSH.

Table 3. Biological outcomes for women treated with gonadotrophin-releasing hormone (GnRH) antagonist or agonist protocol.

<i>Variable per cycle</i>	<i>GnRH antagonist protocol (n = 200)</i>	<i>GnRH agonist protocol (n = 200)</i>
No. of oocytes injected	2.8 ± 0.1	2.9 ± 0.1
No. of normal zygotes ^a	2.6 ± 0.1	2.7 ± 0.1
No. of cleaved embryos	2.6 ± 0.1	2.6 ± 0.1
No. of transferred embryos	2.3 ± 0.1	2.4 ± 0.1
No. of excellent quality embryos	1.0 ± 0.8	1.1 ± 0.9
No. of good quality embryos	1.0 ± 0.9	1.0 ± 0.9
No. of fair quality embryos	0.3 ± 0.4	0.3 ± 0.5
Overall pregnancy rate (%)	33.8	34.7
Clinical pregnancy rate (%)	28.4	30.3
Implantation rate (%)	17.6	18.7

^aZygotes with two equal-sized pronuclei detected 12–16 h after intracytoplasmic sperm injection.

Values are mean ± SD, unless otherwise stated. There were no statistically significant differences between the two groups.

pregnancy and implantation rate between women treated with GnRH antagonist and GnRH agonist regimens (**Table 3**). In addition, clinical pregnancy occurred in two (20%) women in whom the GnRH antagonist-based stimulation was delayed due to high progesterone concentrations.

The percentage of women who had at least one adverse experience during the treatment period was similar in both groups. In the GnRH agonist group, the most frequently reported complaints were headache, spotting and abdominal pain, whereas in the GnRH antagonist group abdominal pain was the only side-effect mentioned. The occurrence of ovarian hyperstimulation syndrome was rare, and none

of the patients required hospitalization. Regarding the patients' opinion about the ovarian stimulation modality, many women complained of a long treatment duration, and in consequence more frequent medical controls and drug injections in the GnRH agonist regimen, whereas in the GnRH antagonist regimen, some patients expressed their disappointment with the high cost of GnRH antagonist (data not shown).

Overall patient satisfaction with the treatment regimen was different in both study groups, with moderate to great satisfaction expressed by 72% and 85% in the buserelin and ganirelix groups ($P < 0.01$) respectively.

Discussion

The results of this case-controlled study, undertaken in the context of a ban on fertilization of more than three oocytes and performed on a population of women under 40 years with normal ovarian reserve, showed that cycles stimulated with the use of the GnRH antagonist were of shorter duration, required a lower total FSH dose, achieved lower peak oestradiol concentrations and yielded fewer total and metaphase II oocytes compared with cycles stimulated with GnRH agonist in the long protocol. These findings are in agreement with previously published observations (Albano *et al.*, 2000; Born and Mannarets 2000; Olivennes *et al.*, 2000; European and Middle East Orgalutran Study Group 2001; Ludwig *et al.*, 2001; Al-Inany *et al.*, 2006; Kolibianakis *et al.*, 2006). However, no significant differences in the number of normally fertilized oocytes, cleaved embryos, and the pregnancy and implantation rates were found between the two ovarian stimulation protocols.

The findings of a similar clinical outcome from the population of young women undergoing GnRH antagonist- and agonist-based ovarian stimulation cycles demonstrate that GnRH antagonists applied in the group of women under 40 years with normal ovarian reserve do not further reduce the clinical success of IVF programmes just slightly compromised by the intrinsic characteristics of a new policy (Ragni *et al.*, 2005). Moreover, this experience clearly contrasts the growing trend to consider the use of GnRH antagonists as a second option, applied by exclusion in patients with unfavourable *a priori* prognosis *a priori* or after previous poor results with GnRH agonists (Nicoletto *et al.*, 2001; Fasouliotis *et al.*, 2003; Griesinger *et al.*, 2006).

In addition, the high overall satisfaction with the GnRH antagonist protocol execution, expressed by a majority of the women, should lead to consideration of this regimen as particularly attractive in terms of social and medical costs.

However, until now, the application of GnRH antagonists in IVF programmes has developed slowly due to clinical inertia and a perception of lower pregnancy rates (Al-Inany *et al.*, 2002, 2006; Aboulghar 2004). In fact, looking at the study population, even if the clinical results are satisfactory, a better pregnancy rate has not been achieved in cycles using GnRH antagonists as compared with those based on GnRH agonists. That is why, in compliance with the patients' increasing demand for friendly protocols, every effort should be made to uncover why pregnancy rates are slightly reduced in the GnRH antagonist cycles.

The present study was performed with the restriction of the number of oocytes subjected to ICSI to a maximum of three, in accordance with the conditions imposed by Italian law, and thus gives the opportunity to evaluate the possible causes of reduced clinical success in GnRH antagonist cycles in a new light.

The most important step in understanding the interference between GnRH antagonists and IVF programme results is acknowledgment that GnRH antagonist are potent inhibitors of the cell cycle, decreasing the synthesis of locally produced growth factors. They are able to exhibit this activity in all

tissues presenting GnRH receptors, and consequently influence blastomere formation, endometrium development, and, finally, folliculogenesis and oocyte maturation (Ortmann and Diedrich, 1999; Hernandez, 2000).

As to the first question, it is possible to exclude a detrimental effect of GnRH antagonists on embryo development, as no differences were found in the number of cleaved or transferred embryos and their morphological quality in both groups of treatment.

As to the second point, the present findings do not allow confirmation of the hypothesis that alteration of the uterine environment occurs in cycles using GnRH antagonists. In fact, if endometrial receptivity were compromised with the use of GnRH antagonists, as suggested (Saadat *et al.*, 2004; Kolibianakis *et al.*, 2005), the restriction of the oocyte number to three could be expected to exacerbate this disadvantage by adding another negative factor; restriction of the number of transferable non-selected embryos.

Notwithstanding this, in GnRH antagonist-based cycles, the elimination of other factors potentially negative for endometrial receptivity, such as elevated progesterone concentrations at the beginning of ovarian stimulation, remains fundamental (Kolibianakis *et al.*, 2004). The observation of only slightly lower pregnancy rates in women delayed because of initially elevated progesterone concentrations is in contrast with the previous suggestion that cycles with high day 2 progesterone concentrations should be cancelled (Kolibianakis *et al.*, 2004). This discrepancy may be casual, due to the low number of patients showing at least only a subtle progesterone increment. Although no endometrial histological data are available in this study, it seems reasonable to exclude the role of endometrial/embryonic factor. It is important to underline that in the present study, all women underwent prolonged and strong luteal support with a natural micronized progesterone.

In view of these findings and interpretations, the main cause of the previously reported trend towards lower clinical success in ovarian stimulation using GnRH antagonists is likely to be related to the quantitative and qualitative characteristics of oocytes retrieved in these cycles.

On a physiological basis, the exogenous FSH dose, together with a high endogenous FSH concentration normally present during the inter-cycle phase, should guarantee excellent follicular recruitment (Fauser and Van Heusden, 1997), but the number of oocytes retrieved with GnRH antagonists is lower compared with the long GnRH agonist protocol (Albano *et al.*, 2000; Born and Mannarets 2000; Olivennes *et al.*, 2000; European and Middle East Orgalutran Study Group 2001; Ludwig *et al.*, 2001). In fact, it has been suggested that GnRH antagonists may increase the number of follicles arrested in the primordial stage (Ganirelix Dose Finding Study Group, 1998; Felberbaum and Diedrich, 1999).

Therefore, the limited number of oocytes used in both treatment groups before the execution of ICSI seems to counteract the difference in pregnancy rate between these two protocols by eliminating the advantage associated with a higher number of fertilizable oocytes, and thus a better embryo selection, when legally allowed, in the long protocol.

Interestingly, it has been demonstrated that a higher starting dose of FSH may increase the number of oocytes, but it does not appear to be associated with a higher pregnancy rate (Wilkland *et al.*, 2001; Out *et al.*, 2004), just as the increase of gonadotrophin dose at GnRH antagonist initiation does not seem to result in a higher probability of pregnancy (Aboulghar, 2004). This apparent incapacity to improve clinical success even in the presence of substantially greater numbers of oocytes strengthens the possibility of lower oocyte quality in GnRH antagonist-based cycles. In fact, it has been suggested that GnRH antagonist may interfere negatively with follicular granulosa cell division and the cell signalling pathway, driving the oocyte into metaphase II (MII) (Shirahige *et al.*, 1994; Pinski *et al.*, 1996).

Therefore, the finding that there is no difference in oocyte fertilization and the number of cleaved embryos can be ascribed to oocyte selection, because it has been shown that visualization of the meiotic spindle in MII oocytes and its position with regard to the location of the first polar body at the time of ICSI has a predictive value of the risk of abnormal fertilization (Rienzi *et al.*, 2003). This novel approach seems to improve the clinical efficacy of cycles using GnRH antagonists by eliminating those oocyte abnormalities that can be at least partly responsible for impaired ICSI outcome, so further reducing the number of transferable embryos.

The number and quality of oocytes recovered from GnRH antagonist-based protocols are associated with a shorter duration of stimulation, a lower FSH dose and a lower oestradiol peak, conditions which are related to a reduction of discomfort, cost and health risk associated with the treatment. Thus, the slightly deficient level of oocyte retrieval, with its consequence for embryo selection capacity (when possible) has to be considered a price to be paid for making the ovarian stimulation protocol more friendly. However, the application of recently published recommendations concerning the optimization of GnRH antagonist ovarian stimulation protocols, including cycle delay or cancellation in patients with initially elevated oestradiol and progesterone concentrations (Kolibianakis *et al.*, 2004), avoiding prolonging the follicular phase by early administration of HCG (Kolibianakis *et al.*, 2005), prolonging luteal phase support (Kolibianakis *et al.*, 2004), and, based on present experience, active oocyte selection, allow for improvement of the pregnancy rate from these cycles even if ICSI has to be performed with a reduced number of oocytes.

This study draws attention to the previously published commentary (Sauer, 2004) suggesting that the use of scientific advancement in medical practice allows the achievement, in some patients, of reasonable success rates even inside the jurisdiction of law 40/2004. However, there is still no legally acceptable solution for many specific problems, and it is hoped that the Italian restrictions are never applied elsewhere.

In summary, the data demonstrate that, in the conditions of legal restriction of the number of oocytes used in ICSI, in young women with normal ovarian reserve there is no difference in terms of clinical outcome between protocols using GnRH antagonists or GnRH agonists. Undoubtedly, more research should be performed to determine the real mechanism of GnRH antagonist interference with IVF results, but based on current experience, their involvement in depressing the processes

of follicular recruitment and maturation seems to be more plausible than involvement in compromising endometrial receptivity. However, it is clearly evident that application of some laboratory, medical and biological regimens, including the pioneering strategy of oocyte selection, can improve pregnancy rate in cycles using GnRH antagonists. Although further, and possibly randomised, studies are necessary to confirm these results and draw any definitive conclusions regarding the use of GnRH antagonist in Italian reproductive medicine, the present findings encourage their promotion to young women as an efficacious, safe and minimally invasive alternative to GnRH agonist in a gold standard long protocol.

References

- Aboulghar MA 2004 Meta-analysis of RCTs comparing GnRH-agonist and GnRH-antagonist. Abstract of the *ESHRE Campus Symposium 2004 on GnRH-antagonist in IVF*, p. 13.
- Albano C, Felderbaum RE, Smith J *et al.* 2000 Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. *Human Reproduction* **3**, 526–531.
- Al-Inany HG, Abou-Setta AM, Aboulghar M 2006 Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Systematic Review* **19**, 3. CD001750.
- Al-Inany HG, Aboulghar MA, Mansour RT *et al.* 2005 Optimizing GnRH antagonist administration. Meta-analysis of fixed versus flexible protocol. *Reproductive BioMedicine Online* **10**, 567–570.
- Benagiano G, Gianaroli L 2004 The new Italian IVF legislation. *Reproductive BioMedicine Online* **9**, 117–125.
- Born G, Mannaerts B 2000 Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe, convenient: results of a controlled, randomized, multicentre trial. The Orgalutran Study Group. *Human Reproduction* **7**, 1490–1498.
- Cohen J 2006 Procreative tourism and reproductive freedom. *Reproductive BioMedicine Online* **13**, 145–146.
- European and Middle East Orgalutran Study Group 2001 Comparable clinical outcome using the GnRH-antagonist ganirelix or a long protocol of the GnRH-agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. *Human Reproduction* **16**, 644–651.
- Fasoulitis SJ, Laufer N, Sabbagh-Ehrlich S *et al.* 2003 Gonadotrophin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. *Journal of Assisted Reproductive Genetics* **20**, 455–460.
- Fauser BC, Van Heusden AM 1997 Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocrinology Review* **18**, 71–106.
- Felberbaum RE, Diedrich K 1999 Ovarian stimulation for IVF/ICSI with gonadotrophins and GnRH analogues: agonist and antagonist. *Human Reproduction* **14**, 207–221.
- Fluker M, Grifo J, Leader A *et al.* 2001 Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. *Fertility and Sterility* **75**, 38–45.
- Ganirelix Dose-Finding Study Group 1998 A double-blind, randomized, dose finding study to assess the efficacy of the GnRH-antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). *Human Reproduction* **13**, 3023–3031.
- Griesinger G, Diedrich K, Tarlatzis BC, Kolibianakis EM 2006 GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome and

- risk of ovarian hyperstimulation: a meta-analysis. *Reproductive BioMedicine Online* **13**, 628–638.
- Hernandez ER 2000 Embryo implantation and GnRH antagonists: embryo implantation: the Rubicon for GnRH antagonists. *Human Reproduction* **15**, 1211–1216.
- Kolibianakis EM, Collins J, Tarlatzis BC *et al.* 2006 Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Human Reproduction Update* **12**, 651–671.
- Kolibianakis EM, Bourgain C, Papanikolaou EG *et al.* 2005 Prolongation of follicular phase by delaying HCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. *Human Reproduction* **20**, 2453–2456.
- Kolibianakis EM, Zikopoulos K, Smitz J *et al.* 2004 Elevated progesterone at initiation of stimulations associated with lower ongoing pregnancy rate after IVF using GnRH antagonists. *Human Reproduction* **19**, 1525–1529.
- Ludwig M, Katalinic A, Diedrich K 2001 Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. Meta-analysis. *Archives of Gynecology and Obstetrics* **265**, 175–182.
- Nicolettos N, Al-Hasani S, Felberbaum R *et al.* 2001 Gonadotrophin-releasing hormone antagonist protocol: a novel method of ovarian stimulation in poor responders. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **97**, 202–207.
- Olivennes F, Belaisch-Allart J, Emperaire JC *et al.* 2000 Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LHRH) antagonist (cetorelix) or a single depot formula of a LHRH agonist (triptorelin). *Fertility and Sterility* **73**, 314–320.
- Ortmann O, Diedrich K 1999 Pituitary and extrapituitary actions of gonadotrophin-releasing hormone and its analogues. *Human Reproduction* **14**, 194–206.
- Out HJ, Rutherford A, Fleming R *et al.* 2004 A randomized, double-blind, multicentre clinical trial comparing starting doses of 150 and 200 IU of recombinant FSH in women treated with the GnRH antagonist ganirelix for assisted reproduction. *Human Reproduction* **19**, 90–95.
- Pinski J, Lamharzi N, Halmos G *et al.* 1996 Chronic administration of the luteinizing hormone-releasing hormone (LHRH) antagonist Cetorelix decreases gonadotrophe responsiveness and pituitary LHRH receptor messenger ribonucleic acid levels. *Endocrinology* **137**, 3430–3436.
- Ragni G, Allegra A, Anserini P *et al.* 2005 The 2004 Italian legislation regulating assisted reproduction technology: a multicentre survey on the results of IVF cycles. *Human Reproduction* **20**, 2224–2228.
- Rienzi L, Ubaldi F, Martinez F *et al.* 2003 Relationship between meiotic spindle location with regard to the polar body position and oocyte developmental potential after ICSI. *Human Reproduction* **18**, 1289–1293.
- Saadat P, Boostanfar R, Slater CC *et al.* 2004 Accelerated endometrial maturation in the luteal phase of cycles utilizing controlled ovarian hyperstimulation: impact of gonadotropin-releasing hormone agonists versus antagonists. *Fertility and Sterility* **82**, 167–171.
- Sauer MV 2006 Italian law 40/2004: a view from the 'Wild West'. *Reproductive BioMedicine Online* **12**, 8–10.
- Scheffer GJ, Broekmans FJ, Dorland M *et al.* 1999 Antral follicle count by transvaginal sonography are related to age in women with proven natural fertility. *Fertility and Sterility* **72**, 845–851.
- Shirahige Y, Cook CB, Pinski J *et al.* 1994 Treatment with luteinizing hormone-releasing hormone antagonist SB-75 decrease levels of epidermal growth factor receptor and its mRNA in OV-1063 human epithelial ovarian cancer xenografts in nude mice. *International Journal of Oncology* **5**, 1031–1035.
- Smotrich DB, Widra EA, Gindoff PR *et al.* 1995 Prognostic value of day 3 estradiol on in vitro fertilization outcome. *Fertility and Sterility* **64**, 1136–1140.
- Tarlatzis BC, Fauser BC, Kolibianakis EM *et al.* 2006 GnRH antagonists in ovarian stimulation for IVF. *Human Reproduction Update* **12**, 333–340.
- Tesarik J, Greco E 1999 The probability of abnormal preimplantation development can be predicted by a single static observation of pronuclear stage morphology. *Human Reproduction* **14**, 1318–1323.
- Tesarik J, Greco E, Mendoza C 2001 Assisted reproduction with in-vitro-cultured testicular spermatozoa in cases of severe germ cell apoptosis: a pilot study. *Human Reproduction* **16**, 2640–2645.
- Wilklund M, Bergh C, Borg K *et al.* 2001 A prospective, randomized comparison of two starting doses of recombinant FSH in combination with cetorelix in women undergoing ovarian stimulation for IVF/ICSI. *Human Reproduction* **16**, 1676–1681.

Received 1 December 2006; refereed 20 December 2006; accepted 16 March 2007.