Pituitary down-regulation in IVF/ICSI: consequences for treatment regimens

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Chapter 2

The effect of an individualized GnRH antagonist protocol on folliculogenesis in IVF/ICSI

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Background

The aim of this study was to assess the effect of an individualized GnRH antagonist regimen on folliculogenesis.

Methods: In a multicentre, randomized, clinical trial, IVF/ICSI patients were allocated to a standard regimen, in which they received daily 0.25 mg GnRH antagonist ganirelix (Orgalutran) from the 6th day of stimulation onward (fixed regimen *n*=102) or to an individualized regimen, in which IVF/ICSI patients received daily 0.25 mg GnRH antagonist starting on the day that the dominant follicle had reached a diameter of $\geq$15 mm (flexible regimen *n*=103). The primary endpoint was to assess the difference in the total number of oocytes.

Results: The mean (SD) number of retrieved oocytes was not statistically significantly different: 9.4 (5.8) in the flexible group versus 9.7 (6.5) in the fixed group. The clinical and ongoing pregnancy rates were 22.7 and 21.8% respectively in the flexible group versus 33 and 31.1% in the fixed group [relative rate ratio 0.69 (95% confidence interval 0.44–1.08) and 0.7 (0.44–1.12) respectively.

Conclusion: The individualized flexible regimen did not result in an increase in the total number of oocytes obtained.

Introduction

Since the introduction of GnRH agonists, the results of controlled ovarian stimulation (COS) in IVF have greatly improved. The cancellation rate is decreased by the prevention of premature LH surges, follicular recruitment is enhanced, and the ovarian response to hyperstimulation is better synchronized, thus facilitating the scheduling for oocyte retrieval. (Caspi *et al.*, 1989; Vauthier and Lefebre 1989; Ron-El *et al.*, 1991; Hughes *et al.*, 1992). The introduction of the safe and effective third generation of GnRH antagonists provided an alternative to the GnRH agonists. Unlike the indirect form of pituitary suppression by GnRH agonists, which requires ≥2 weeks of administration, the GnRH antagonists cause an immediate and direct suppression by competitive binding with the GnRH receptors. GnRH antagonists can therefore be administered just before the expected LH surge and need to be administered only for a few days. In most studies, the GnRH antagonist was administered on stimulation day 6 of recombinant FSH, and the average duration of GnRH administration was 4–5 days (Albano *et al.*, 1997; European Orgalutran Study Group, 2000; European Middle East Orgalutran Study Group, 2001).

COS, in combination with GnRH antagonists, has proved to be equally successful in preventing premature LH surges as GnRH agonists and cancellation rates have been comparable. The number of retrieved oocytes and estradiol levels are lower, which might have a beneficial effect on the occurrence of ovarian hyperstimulation syndrome (OHSS) in GnRH antagonist regimens (Al-Inany and Alboulghar, 2002). Combined with the patient-friendly aspects, GnRH antagonist appeared to become the treatment of choice. However, GnRH antagonist-treated patients showed lower clinical pregnancy rates compared to GnRH agonist-treated patients (odds ratio 0.79, 95% CI 0.63–0.99) (Alinany and Alboulghar, 2002).

There is some evidence that individualizing or tailoring the GnRH antagonist administration would lead to better clinical results. This flexible regimen was described previously and seemed to be able to prevent LH surges adequately (Olivennes *et al.*, 1995). A randomized trial assessed the number of days of GnRH antagonist administration and the number of monitoring visits of a flexible regimen in which the GnRH administration was delayed until the dominant follicle reached ≥15 mm in diameter. It was suggested that this flexible regimen led to a higher number of retrieved oocytes and improved clinical outcome. (Ludwig *et al.*, 2002). This study, however, was not powered

on oocyte retrieval and therefore not suited for drawing definite conclusions.

The aim of this randomized clinical trial was to assess, with sufficient power, whether an individual regimen of GnRH antagonist, started when the lead follicle measures ≥15 mm, yields more oocytes than a fixed regimen of the GnRH antagonist started on day 6 of ovarian stimulation.

Materials and Methods

Seven fertility clinics in The Netherlands participated in this randomized clinical trial between April of 2001 and October 2002. The study was approved by the Institutional Review Boards of all clinics. Eligible patients with an indication for IVF/ICSI treatment were recruited. Inclusion criteria were subject ages between 18 and 39 years, body mass index between 18 and 29 kg/m^2 and body weight between 50 and 90 kg, and a normal menstrual cycle with a range of 24–35 days with an intra-individual variation of 3 days. Patients with a contraindication for the use of GnRH antagonists, polycystic ovarian syndrome, ovarian cysts, a history of low response to FSH treatment, a history of ovariectomy, and more than three previous IVF/ICSI attempts were excluded from the trial. After written informed consent, patients were allocated to an individualized regimen in which IVF/ICSI patients received daily 0.25 mg GnRH antagonist (ganirelix: Orgalutran®; Organon, The Netherlands) starting on the day that the dominant follicle reached a diameter of ≥15 mm (flexible regimen) or the standard regimen in which the GnRH antagonist was given from stimulation day 6 onwards (fixed regimen). The GnRH antagonist was administered s.c. in the upper leg before 10:00, after ultrasound and blood sampling.

Randomization was performed using sealed opaque envelopes prepared by a third party, Clinical Trial Operations BV hired by Organon NV, The Netherlands. Blocking was done per study centre, and the envelopes were numbered randomly. At the first visit on cycle days 1 or 2, a transvaginal ultrasound was performed to exclude cysts. Thereafter the first envelope, by number, was opened by the physician of the clinic involved, in the presence of the patient. On the second or third day, multiple follicular development was induced with a fixed dose of 200 IU of recombinant (r)FSH (Puregon®; Organon, The Netherlands) for 5 days and individually adjusted thereafter, if necessary. Patients were instructed to administer the rFSH daily in the afternoon s.c. in the abdominal wall. Monitoring was performed by ultrasound after 5 days of rFSH stimulation (stimulation day 5) in the flexible group, in the fixed group after 6

days of rFSH stimulation (stimulation day 6), and every other day thereafter. The last ultrasound was performed on the day of hCG administration. The total numbers of follicles ≥11, ≥15 and ≥18 mm were counted. Hormone measurement was assessed for 17β-estradiol (E_2) (pg/ml) and LH (IU/l). When at least three follicles measured ≥18 mm in diameter, patients received their last GnRH antagonist injection in the morning and final follicular maturation was induced the same evening by 10 000 IU hCG (Pregnyl®; Organon, The Netherlands). Oocyte retrieval took place 36 h after hCG administration. Retrieved oocytes were classified as metaphase II oocytes, metaphase I oocytes, germinal vesicle stage for ICSI patients, or Score 1, 2 or 3 (atretic oocytes) for IVF patients (Veeck, 1988). Embryos were classified according to fixed criteria (Puissant *et al.*, 1987; Veeck, 1988): Score I, excellent quality, no fragmentation; Score II, good, 1–10% fragmentation; Score III, fair, 21–50% fragmentation.

Embryo transfer followed 72 h after oocyte retrieval. A maximum of two embryos were transferred and surplus embryos, when eligible, were stored frozen. Luteal support was given according to the local protocol of each centre.

Analysis

The primary endpoint of this study was to assess a difference in the total number of retrieved oocytes between both groups. The secondary endpoints were the number of cancellations; the number of days and total dose of rFSH administration; duration of GnRH antagonist administration; premature LH surges; follicle growth; quality of oocytes; fertilization rate; number and quality of obtained, transferred and frozen embryos; and clinical and ongoing pregnancy rates. Clinical pregnancies were defined as intrauterine pregnancy with at least one fetus with positive heartbeat by ultrasound at 6 weeks after embryo transfer. Ongoing pregnancies were defined as a positive fetal heartbeat by ultrasound at 10 weeks after embryo transfer.

The study was designed as a superiority trial with respect to oocyte yield. Considering an oocyte yield of eight in the fixed group, with an alpha of 5% and a beta of 20%, and considering an oocyte yield of 10 in the flexible group as a clinically significant difference, 200 patients were required. The analysis of all outcomes was on an intent-to-treat basis.

All serum samples were frozen and stored at –20°C. When the collection of blood samples was completed, the hormonal analyses were performed by a central laboratory (ABL, Assen, The Netherlands) with an radioimmunoassay

assay within one batch. The intra-assay variation was 5% for LH and E_2 respectively.

Student's *t*-test or x^2-tests were performed, as appropriate. Pregnancy outcomes were expressed as a relative pregnancy rate ratio (RR), with corresponding 95% confidence intervals (CI). Statistical significance was defined as $P<0.05$. For the secondary outcomes, conventional significance levels do not apply, and we defined, according to the Bonferroni correction, $P=0.005$ as statistically significant.

Results

Two hundred and five patients were randomized, 102 to the flexible regimen and 103 to the fixed regimen. One patient in the flexible group failed to administer rFSH and was excluded from the trial. The number of patients randomized, treated and undergoing oocyte retrieval and embryo transfer is presented in Figure 1.

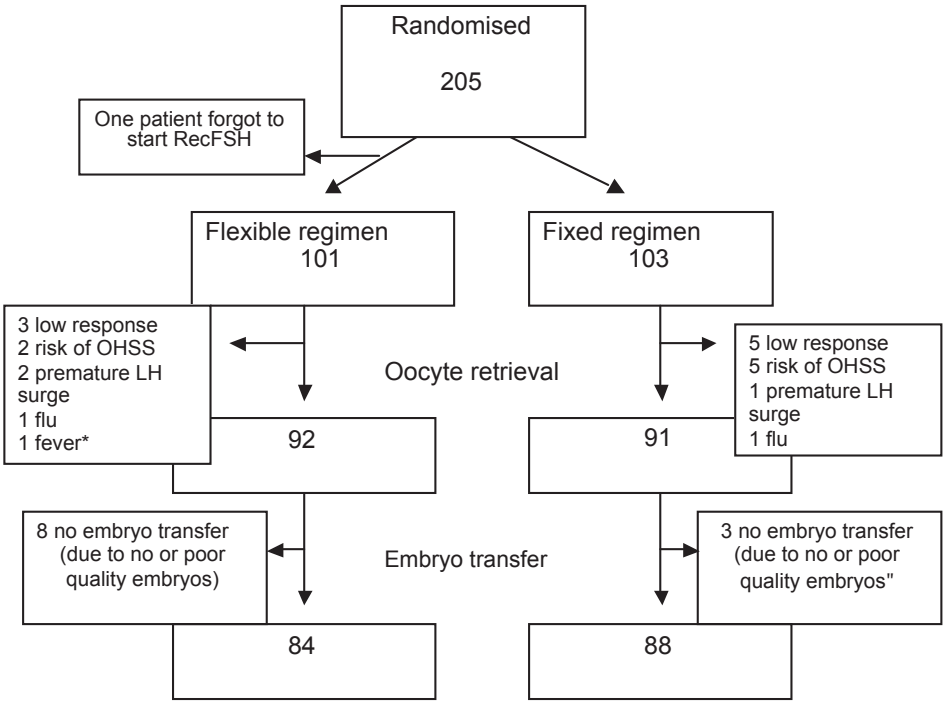

Fig.1 flow chart on subject disposition

Baseline characteristics and gynaecological history are summarized in Table I. Age, body mass index, duration of infertility, parity, and sperm count were equally divided between the two groups, as were the causes of infertility. In total, 51 patients had ICSI and 131 patients had IVF.

Table I. Patient demographics and gynaecological history

	Flexible regimen		Fixed regimen	
	N	(SD*)	N	(SD)
Mean age (year)	33.1	(3.6)	33.0	(3.4)
BMI (kg/m2)	23.0	(2.7)	22.9	(2.6)
Duration of infertility (year)	3.1	(1.6)	3.6	(2.0)
Primary infertility (%)	56.4		55.3	
IVF	72		81	
ICSI	29		22	
Indication IVF/ICSI (%)				
Male factor	41.6		39.8	
Tubal factor	17.8		17.5	
Unexplained	27.7		28.2	
Endometriosis	3		3.9	
Cervical factor	3		0	
Other	3		5	
Total Motile Sperm Count (TCM)	69 (97)		64 (76)	

*SD = standard division

The results are summarized in Table II. We found no differences in the total days and total amount of rFSH administration. In the flexible group, the mean GnRH antagonist administration starting day was stimulation day 7. In the flexible group, 25 patients started the GnRH antagonist administration on stimulation day 5 (one on day 4), 20 patients on stimulation day 6, 33 patients on day 7, eight patients on day 8 and 13 patients on day 9. In the fixed group, all patients, except one, started on stimulation day 6. The number of days of administration of GnRH antagonist were 4.7 in the flexible group and 5.2 in the fixed group (P=0.025). On average, hCG was administered on stimulation day 10 in both groups, varying from stimulation days 7 to 18 and 6 to 16 for the flexible group and fixed group respectively. Premature luteinization was observed in one patient in the fixed group (1.0%) and two patients in the flexible group (2.0%). No differences were found in the quality and the mean number of retrieved oocytes. Fertilization rate was the same in both groups,

as well as the quality and total number of obtained, transferred, and frozen embryos.

Table II. IVF/ICSI results

	Flexible regimen	Fixed regimen	*P*
rFSH treatment (days)[a]	9.4 (2.0)	9.4 (1.6)	NS
Total amount of rFSH (IU)[a]	1924 (525)	1879 (406)	NS
Total no. of patients who started GnRH antagonist on stimulation day:			
4/5	25		
6	20	102	
7	33	1	
8	8		
9/10	13		
Missing values	2	0	
GnRH antagonist treatment days	4.7	5.2	0.025
Premature LH surge	2	1	
Mean stimulation day of hCG	10.5	10.6	
Mean no. of oocytes retrieved[b]	9.4 (5.8)	9.7 (6.5)	NS
Quality of oocytes[ab]			
ICSI results	*n*=29	*n*=22	
No. of metaphase II oocytes[a]	9.1 (5.5)	8.8 (5.0)	NS
No. of metaphase I oocytes[a]	0.5 (0.8)	0.6 (1.0)	NS
No. of germinal vesicles[a]	1.2 (1.5)	0.8 (1.0)	NS
IVF results	*n*=63	*n*=68	
Score I	3.9 (4.4)	4.9 (5.8)	NS
Score II	1.7 (2.4)	2.2 (3.5)	NS
Score III	0.1 (0.4)	0.2 (0.8)	NS
Fertilization rate (%)	56.7 (26.9)	57.3 (26.5)	NS
Quality of embryos[c]			
Grade I	0.9 (1.6)	1.4 (2.7)	NS
Grade II	2.5 (2.8)	2.9 (3.0)	NS
Grade III	2.2 (2.5)	2.0 (2.6)	NS
Total no. of embryos transferred	1.9 (0.3)	1.9 (0.3)	NS
Total no. (%) of embryos frozen	1.5 (3.3)	1.2 (2.2)	NS
No. (%) of clinical pregnancies[b]	23 (22.7)	34 (33.0)	NS
No. (%) of ongoing pregnancies	22 (21.8)	32 (31.1)	NS
Twins	4	8	
No. (%) of ongoing pregnancies/GnRH antagonist start day			
start day:	ongoing pregnancies: (%)		
4/5	9 (36)		
6	4 (20)	32 (31.1)	
7	8 (24.2)		
8	1 (12.5)		
9/10	0 (0)		

[a] Mean (SD). [b] Per recombinant (r)FSH started cycle. NS = not significant.

Although not statistically significant, fewer clinical and ongoing pregnancies were found in the flexible group as compared to the fixed group; 22.7 versus 33.0% for clinical pregnancy (RR 0.69, 95% CI 0.44–1.08) and 21.8 versus 31.1% for ongoing pregnancy (RR 0.70, 95% CI 0.44–1.12) respectively. In total, four twin pregnancies and one ectopic pregnancy occurred in the flexible group and eight twins in the fixed group. To assess the impact on pregnancy rate of the stimulation day on which the GnRH antagonist administration was started, the different pregnancy rates were calculated for each stimulation day separately. Although the numbers are very small, there seems to be a declining trend in pregnancy rate in the flexible group from stimulation day 8 onward.

The follicular growth pattern during the GnRH antagonist administration is presented in Table III. At the first ultrasound, performed on the first day of GnRH antagonist administration, we found, although not statistically significant, more follicles of ≥11 and ≥15 mm in the flexible group as compared to the fixed group. This difference became more distinct when the different stimulation days on which the GnRH antagonist was started were assessed separately. From stimulation day 7 onward, a trend towards more follicles of ≥11 and ≥15 mm was seen. At the last ultrasound, at the end of the stimulation phase, the size and number of the follicles were the same in both groups.

Table III. Follicle growth pattern

	Flexible regimen				**Fixed regimen**				***P***
	n	No. of follicles (SD)			*n*	No. of follicles (SD)			
		≥11 mm	≥15 mm	≥18 mm		≥11 mm	≥15 mm	≥18 mm	
First ultrasound (GnRH start)	99[a]	7.1 (4.7)	2.1 (2.9)	0	102[b]	5.8 (4.0)	1.1 (1.4)	0	NS
Stimulation day									
4/5	25	4.7	1.6	0	0				
6	20	5.2	1.2	0	102	5.8	1.1	0	
7	33	8.0	2.1	0	1				
8	8	8.1	2.0	0	0				
9/10	13	11.8	5.2	0	0				
Last ultrasound	101	12.0 (7.5)	8.9 (6.6)	4.6 (3.5)	103	12.9 (7.5)	8.8 (5.4)	4.5 (3.8)	NS

[a] No. of missing values = 2. [b] No. of missing values = 1. NS = not significant.

Table IV. Median serum hormone values

	n	Flexible	Fixed	*P*
17 -Estradiol (pg/ml)				
On first day of GnRH antagonista		522 (148–2140)	461 (144–1340)	NS
No. of patients on stimulation day				
4/5	25	336		
6	20	563		
7	33	584		
8	8	923		
9/10	13	1080		
On day 3 of GnRH antagonista		921 (348–2880)	899 (242–2980)	NS
End of stimulation phasea		1310 (341–3910)	1620 (667–3970)	NS
LH values (IU/l):		n=95	n=94	
First day of GnRH antagonista		2.7 (0.7–12.7)	3.7 (0.7–14.4)	NS
On day 3 of GnRH antagonista		1.5 (0.6–5.1)	1.9 (0.6–4.7)	NS
End of stimulation phase		1.3 (0.6–5.4)	1.8 (0.6–7.0)	NS

[a] Median (5th–95th percentile). NS = not significant.

Serum hormone values are presented in Table IV. No significant differences were found between the two groups, but it should be noticed that the median E_2 level on the first and third days of GnRH antagonist administration was to some extent higher in the flexible group compared to the fixed group. The difference in E_2 values increased especially in the group of patients who received their first GnRH antagonists on stimulation days 8, 9 and 10, whereas at the end of the stimulation phase, the E_2 levels were, although not statistically significant, to some extend lower in the flexible group compared with the fixed group. The median LH levels were comparable between both treatment groups

Lastly, a subanalysis was performed on the presence or absence of a follicle ≥15 mm on stimulation day 6, in relation to the ongoing pregnancy rate (Table V). The ongoing pregnancy rate in patients with a follicle ≥15 mm on stimulation day 6 was 47.0% in the fixed group versus 26.7% in the flexible group. In patients without a follicle ≥15 mm on stimulation day 6, the pregnancy rates were 15.6% in the fixed group versus 17.8% in the flexible group.

Table V. Subanalysis on presence or absence of a follicle ≥15 mm on stimulation day 6

	Patients *n*	Ongoing pregnancies *n* (%)	Median 17 -estradiol (pg/ml)
Fixed starting group			
Follicles ≥15 mm present	51	24 (47.0)	703
Follicles ≥15 mm absent	51	8 (15.6)	414
Flexible starting group			
Follicles ≥15 mm present	45	12 (26.7)	409
Follicles ≥15 mm absent	54	10 (17.8)	643

Discussion

This is the first adequately powered randomized study to compare the oocyte yield of an individualized GnRH antagonist regimen with the standard fixed regimen in IVF/ICSI. We found no difference in the total number of oocytes retrieved in the flexible regimen compared with the fixed regimen. However, despite the equal outcome of all our secondary endpoints, we found a lower (although not statistically significant) pregnancy rate in the individualized flexible regimen.

We tried to identify, within the flexible group, a subgroup of patients who could account for these lower pregnancy rates; we found that, in patients who received their first GnRH antagonist administration on stimulation day 8 or later, the pregnancy rates decreased remarkably. When analysing follicular development and endocrine profiles of these patients, we noticed that they had a higher number of follicles of >11 and <15 mm and a higher E_2 level compared to the patients of the flexible group who received their GnRH antagonist on stimulation day 7 or earlier, and compared to patients of the fixed group who received their first GnRH antagonist administration on stimulation day 6.

In accordance with our findings, a previous similar study found a similar trend towards a lower ongoing pregnancy rate of 24.1% in the flexible group versus 31.1% in the fixed group (Kolibianakis *et al.*, 2003). He identified a subgroup of patients who had no follicle of ≥15 mm on stimulation day 6 and who therefore received the GnRH antagonist at a later stage in the follicular phase. The endocrine profile of these patients revealed a high E_2 level and a high LH level compared to the patients without a follicle of ≥15 mm on stimulation day 6 in the fixed group. It was suggested that the high E_2 and LH levels combined with a delayed start of GnRH antagonist were the cause of the negative effect on pregnancy rate, by means of prolonging the E_2/LH exposure

to the endometrium. Although our findings were similar for the flexible group, we found, unlike Kolibianikis in the fixed group, that patients with high E_2 exposure had better pregnancy rates than patients with low E_2 exposure. We therefore feel that the role of E_2 levels is not completely clarified yet. Intrinsic factors of the patients, e.g. poorer response, may play a role and may influence the results.

As mentioned earlier, a characteristic of the subgroup of patients in the flexible group, who received the GnRH antagonist from stimulation day 8 onward, was the higher number of mid-follicular phase follicles at the start of GnRH antagonist, compared to the fixed group, probably because the cohort of follicles had more time to develop. Previous studies showed that the administration of GnRH antagonists in high dosages can induce an arrest in follicular growth or can even cause atresia (Mais *et al.*, 1986; Fluker *et al.*, 1991; Marshall *et al.*, 1991). This effect of the GnRH antagonist depends not only on the dose and duration of the GnRH antagonist administration but also on the moment of application in the different phases of the menstrual cycle. While the growth and maturation of a dominant follicle of >18 mm is not effected by a sudden LH withdrawal, the mid-follicular phase follicles show a significant decrease in E_2 levels. This may be an indication of an effect on follicular development (Fraser, 1990; Ditkoff *et al.*, 1991; Fluker *et al.*, 1991; Hall *et al.*, 1991. Inherent to the flexible regimen, the GnRH antagonist is started when the majority of follicles reach their most LH sensitive stage, i.e. follicles between >11 and <15 mm.

We therefore feel that delaying the initiation of the GnRH antagonist to the moment the dominant follicle reaches 15 mm, with a well-advanced cohort of follicles is, in fact, too late and may be deleterious for clinical outcome. However, the number of patients in our subgroups is very limited and we want to stress that the interpretation of these data should be made very cautiously.

We pooled the ongoing pregnancy results of our study and three similar previous studies (Ludwig *et al.*, 2002; Kolibianakis *et al.*, 2003; Escudero *et al.*, 2004). A total of 224 patients in the flexible group were analysed versus 218 patients in the fixed group. A Peto odds ratio of 0.68 (95% CI 0.45–1.03) against the flexible regimen was found for ongoing pregnancy rate. This finding seems to confirm that delaying the initiation of GnRH antagonist administration, especially in patients with good ovarian response, is detrimental to the pregnancy outcome. However, in order to prove or refute an increase in pregnancy rate of 24–33%, ≥400 patients are necessary.

Interestingly, a recent study showed that starting the GnRH antagonist on stimulation day 1 compared to stimulation day 6 resulted in an equal follicular development (Kolibianakis *et al.*, 2004). Although this study did not look at the costs and patients' preferences, it seems logical to assume that an early start negatively influences the advantages of GnRH antagonists.

In conclusion, evidence found to date suggests that in a flexible protocol the GnRH antagonist should be initiated earlier. Furthermore, we cannot substantiate the hypothesis that individualized exposure to the GnRH antagonist increases the total number of oocytes obtained. The data thus far presented show that, if a GnRH antagonist is applied in COS, the fixed regimen is the best choice.

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