Timing and duration of use of GnRH antagonist down-regulation for IVF/ICSI cycles have no impact on oocyte quality or pregnancy outcomes

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Abstract

Purpose To evaluate whether oocyte quality, implantation and pregnancy outcomes in in vitro fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI) are related to the duration of gonadotropin-releasing hormone (GnRH)-antagonist use or the timing of its initiation.

Methods Retrospective cohort study of 178 conventional IVF/ICSI cycles. All patients underwent ovarian stimulation with gonadotropins and GnRH-antagonist for pituitary down-regulation. Spearman correlations and logistic regression were used for statistical analysis.

Results There was no correlation between the duration of use or the timing of initiation of GnRH-antagonist with oocyte quality or implantation and pregnancy outcomes. Oocyte quality was influenced by the peak estradiol. Implantation was influenced by the patient's age. Early pregnancy loss, by the endometrial thickness on human chorionic gonadotropin-day. Ongoing pregnancy was independent from the variables evaluated.

Capsule Our study could not confirm the reported detrimental effect of GnRH-antagonists on oocyte quality and endometrial receptivity in IVF/ICSI cycles.

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Conclusions GnRH-antagonist duration of use or starting day did not influence oocyte quality, implantation rates, and pregnancy rates. We hypothesize that a follicle stimulating hormone/luteinizing hormone dose increase when antagonist was started, may have had an impact on our findings.

Keywords GnRH antagonist • Implantation • IVF/ICSI • Oocyte • Pregnancy

Introduction

Results of clinical trials to date suggest that, with gonadotropin releasing hormone (GnRH)-antagonists for pituitary down-regulation, much shorter treatment regimens with fewer injections and possibly less gonadotropin can achieve good clinical results [1–3]. However, even accomplishing similar estradiol levels, ovarian stimulation cycles using GnRH-antagonists down-regulation during in vitro fertilization/intra-cytoplasmic sperm injection (IVF/ICSI) cycles have been reported to have worse pregnancy outcomes when compared to GnRH-agonists [4]. These results prompted the development of numerous small nuances to improve the stimulation outcomes. These concern the type of gonadotropin used, the addition of LH activity after initiation of GnRH, or the ovulation triggering with GnRH-agonists instead of human chorionic gonadotropin (hCG) [5, 6].

GnRH antagonists for pituitary down-regulation during ovarian stimulation have also been shown to directly influence the development of extrapituitary tissues, including the endometrium, the oocyte and the embryo [7]. GnRH antagonists would decrease the effect of endometrial growth factors and thus alter oocyte development and

decrease endometrial receptivity. The half-life of GnRH-antagonists is about 30 h, and embryo transfer is usually performed on day 3 after retrieval [8]. Therefore, the hypothesized effect on the endometrium could still persist at the time of embryo implantation. Also, a potentially direct effect of the GnRH-antagonists on the developing embryo could occur during the same timeframe.

The FDA-approved GnRH-antagonist daily dose for pituitary down-regulation has been set at 0.25 mg subcutaneous injection [1, 2]. The simplest way for physicians to adjust the amount of medication given, and to potentially prevent its direct detrimental effects, is to vary the duration of administration. In our infertility center most patients are down-regulated with GnRH-antagonists and follicle stimulating hormone/luteinizing hormone (LH/FSH) dose is increased at the time of GnRH-antagonist initiation. Our hypothesis was that a longer administration of GnRH-antagonist (in days) would have a negative impact on endometrium, oocyte and embryo development.

Materials and methods

This was a retrospective cohort study of 144 women undergoing 180 conventional IVF/ICSI cycles. All patients underwent ovarian stimulation with gonadotropins (Gonal-F®, Serono, and Repronex®, Ferring) and GnRH-antagonist for pituitary down-regulation (Antagon™, Organon). Down-regulation with GnRH-antagonists is the standard protocol in our institution. Patients were started on oral contraceptives 1 month prior to the stimulation. Oral contraceptives were stopped 2 days before starting ovarian stimulation. Gonadotropins were administered from stimulation day 1 until the day of the hCG trigger following a step-up protocol. GnRH-antagonist (0.25 mg daily) was added from the day when at least one follicle reached 14 mm in diameter and continued until hCG administration. Supplemental LH and FSH (Repronex®), in the form of one ampoule per day, was added to the gonadotropin regimen when GnRH-antagonist was started. HCG was administered when at least two follicles were greater than 18 mm in diameter. Estradiol peak and endometrial stripe were both measured on the day of hCG trigger. The thickest endometrial segment (between the two interfaces of the endometrial–myometrial junction) was measured transvaginally on a ‘frozen’ midplane, longitudinal section of the uterus by two-dimensional ultrasound. Retrieval of the oocytes was performed 36 h after hCG trigger. Embryo transfer was performed on day 3 after retrieval for all the patients except five of them, who had a day 5-transfer.

We used Spearman correlations to predict whether the duration of GnRH-antagonist use, the GnRH-antagonist starting day, or both, would influence oocyte quality,

implantation, and pregnancy rates. We then conducted stepwise forward logistic and stepwise forward multiple regressions with the variables indicative of oocyte quality (‘number of retrieved oocytes’, ‘mature oocytes’, and ‘fertilized oocytes’). ‘Implantation’ (defined as presence of gestational sac/s), ‘ongoing pregnancy’ (defined as pregnancy continuing beyond 28 weeks’ gestation), and ‘early loss’ (defined as miscarriages plus biochemical pregnancies), as dependent variables, were also evaluated.

One pregnancy that ended in elective abortion for mitochondrial disease found after chorionic villous sampling was counted in the overall pregnancy rate, but was excluded from the early pregnancy loss and ongoing pregnancy calculations; two ectopic pregnancies were excluded both from the overall pregnancy rate and the early pregnancy loss calculation (miscarriages plus biochemical pregnancies/overall pregnancy rate=26/94=28%). Two twin and two triplet pregnancies had a vanishing twin/triplet, thus becoming singleton and twin pregnancies, respectively. These last four cases were not included in the number of miscarriages, but were considered as ongoing pregnancies.

In order to evaluate the multiple-cycle effect, the analyses were repeated for the patients who underwent IVF/ICSI cycles more than once ($n=31$ patients, total of 67 cycles), already included in the initial analysis. Additionally, analysis of variance (ANOVA), Chi square and *T*-Test were performed after transforming the ‘duration of GnRH-antagonist’ into a categorical variable. We used Fisher’s exact test for power calculation considering a significance level of 0.05 and a beta error of 80% for the variable ‘implantation’. We used SPSS statistical package for Windows, version 15.0 (SPSS Inc., Chicago, IL).

Results

Table 1 describes the characteristics of the study patients (178 IVF/ICSI cycles). The overall pregnancy rate was 53% (95/178), the ongoing pregnancy rate was 37% (66/177): 46 were singleton, 12 twin, and eight triplet pregnancies. Seventeen of 95 pregnancies were biochemical, defined as a positive serum β -hCG with no gestational sac identified on ultrasound. Nine more pregnancies ended in miscarriage in the first trimester after a gestational sac and/or an embryonal heart rate was detected by ultrasound. Of the singleton pregnancies ending in miscarriage, none of the patients requested a chromosomal analysis of the products of conception. There were no perinatal or infant deaths in our cohort of cases.

With Spearman correlations, neither of our tested variables, ‘duration of GnRH-antagonist administration’ or ‘GnRH-antagonist starting day’, showed a significant

Table 1 Characteristics of the study population ($n=178$ cycles for 142 patients)

Variable	Mean $\pm$ SD	Range
Age (years)	33.8 $\pm$ 4.6	25–45
Day 3 FSH (mIU/mL)	6.2 $\pm$ 2.8	1.0–13
Length of stimulation (days)	11.6 $\pm$ 1.7	7–16
GnRH-antagonist start day	6.9 $\pm$ 1.7	4–10
No. GnRH-antagonist days	4.8 $\pm$ 1.0	3–9
Total FSH dose (IU)	5,175 $\pm$ 3,619	938–17,025
Estradiol on hCG day (pg/mL)	2,407 $\pm$ 962	606–5,668
Endometrial stripe (mm)	10.4 $\pm$ 2.3	5–17
Total no. of follicles >14 mm	10.5 $\pm$ 5.5	3–41
No. oocytes retrieved	14.6 $\pm$ 8.7	1–45
Mature oocytes	11.4 $\pm$ 7.3	1–41
Immature+atretic oocytes	2.4 $\pm$ 2.7	0–24
No. of fertilized oocytes	8.4 $\pm$ 6.5	1–29
No. embryos transferred	2.8 $\pm$ 1.0	1–6
Pregnancy rate (95/178) (%)	53%	–
Implantation rate ^a (%)	21%	–
Early pregnancy loss rate ^b (26/94) (%)	28%	–
Ongoing pregnancy rate (66/177) (%)	37%	–

One pregnancy that ended in elective abortion for mitochondrial disease found after chorionic villous sampling was counted in the overall pregnancy rate, but was excluded from the early pregnancy loss calculation; two ectopic pregnancies were excluded both from the overall pregnancy rate and the early pregnancy loss calculation

^a No. of gestational sacs/no. of embryos transferred per cycle $\times$ 100

^b No. miscarriages plus biochemical pregnancies/total no. pregnancies

correlation with implantation, early loss, ongoing pregnancy, or with the oocyte quality parameters (number of retrieved, number of fertilized, number of mature oocytes).

Logistic regression analysis yielded results as shown in Table 2. For ‘implantation’, the variable ‘age’ was the only negative predictor with an odds ratio (OR)=0.93 (95% confidence interval (CI)=0.86–0.99; $p<0.05$). For the patients who did achieve implantation (of one to three embryos), age was still the only predictor. Of note, neither the starting day of GnRH-antagonist nor the length of ovarian stimulation were predictors of implantation.

For ‘early loss’, regression analysis was conducted excluding and then including the four vanishing twins/triplets. When excluding the vanishing twins/triplets, ‘endometrial thickness’ was the only negative predictor with an odds ratio of 0.78 (95% CI=0.62–0.99) and a $p<$

Table 2 Logistic regression analysis results

Dependent variable	Predictor variable	OR (95% CI)	p -value
Implantation	Age	0.93 (0.86–0.99)	<0.05
Early pregnancy loss	Endometrial thickness	0.78 (0.62–0.99)	<0.05
Ongoing pregnancy	Length of stimulation	0.83 (0.69–1.00)	0.05

0.05. More graphically, for each 1 mm increase in endometrial thickness above the mean 10.4 mm, the risk of early loss decreased by 22%. This effect was noticed up to 12 mm. We couldn’t calculate this effect for further endometrial thickness increases because of the drastically reduced number of cases in these thickness categories. In this model, ‘duration of GnRH-antagonist administration’ and ‘age’ were not predictors of early pregnancy loss. When the vanishing twins/triplets were included in the regression model, endometrial thickness was no longer a predictor of early loss. This was expected, because all four pregnancies continued with either singleton or twin gestations.

When assessing ‘ongoing pregnancy’, the variable ‘length of ovarian stimulation’ was the only negative predictor with an odds ratio of 0.83 (95% CI=0.69–1.00) and a $p<0.05$. More explicated: for each day increase in stimulation length beyond the 12 days mean length, the likelihood of an ongoing pregnancy decreased by 17%.

Multiple regression analyses as shown in Table 3. Examining all the oocyte quality parameters (‘number of retrieved oocytes’, ‘number of fertilized oocytes’, and ‘number of mature oocytes’) we found ‘peak estradiol’ and ‘total FSH dose’ to be the common positive predictors, with $R^2=0.29$ – 0.42 , and $R^2=0.08$ – 0.10 , respectively ($p<0.001$). The total number of follicles >14 mm in diameter was also a third positive predictor for the number of retrieved and the number of mature oocytes with $R^2=0.05$ and $R^2=0.03$, respectively ($p<0.001$).

After transforming the ‘duration of GnRH-antagonist administration’ into a categorical variable, ANOVA (1–9 days of administration), Chi square, and T -test (3–5 versus >6 days of administration) analysis failed to detect an effect of the duration of antagonist use on oocyte quality or pregnancy outcomes among the different groups. A power calculation based on the observed implantation rates (42% and 45% in patients with 3–5 versus >6 days antagonist use) found that we would need nearly 4,000 cycles per group to find a significant difference.

Considering the 31 women who underwent IVF/ICSI for more than one cycle as a group (27 patients underwent two

Table 3 Multiple regression analysis results

Dependent variable	Predictor variable	R^2 change	p -value
No. of retrieved oocytes	Peak E2	0.42	<0.001
	Total FSH dose	0.08	<0.001
	No. follicles >14 mm	0.05	0.001
No. of fertilized oocytes	Peak E2	0.29	<0.001
	Total FSH dose	0.10	<0.001
No. of mature oocytes	Peak E2	0.41	<0.001
	Total FSH dose	0.10	<0.001
	No. follicles >14 mm	0.03	0.001

cycles, three underwent three cycles, and one underwent four cycles), and repeated the same analyses considering the same dependent variables, no significant predictor for any of the pregnancy outcomes was found. For all the oocyte quality parameters ‘peak estradiol’ was found to be the common positive predictor with $R^2=0.47$, 0.49 , and 0.49 , respectively ($p<0.05$). ‘Total gonadotropin dose’ was the second positive predictor for the number of retrieved oocytes ($R^2=0.07$; $p<0.05$); ‘age’ was the second negative predictor for the number of mature oocytes ($R^2=0.08$; $p<0.05$).

Discussion

When using GnRH-antagonist for pituitary down-regulation, the duration of GnRH-antagonist administration in days and the timing of initiation in the ovarian stimulation were of no value in predicting oocyte quality or pregnancy outcomes. Other studies found a difference with a smaller number of patients than in our study. We speculate that supplementation with LH activity and FSH, implemented in our patients, may have had a positive bearing on both oocyte quality and endometrial effects [5, 6]. In this regard, our results confirm Acevedo et al. study [5]. The authors randomly assigned 20 oocyte donors to ovarian stimulation protocols using GnRH-antagonist alone or GnRH-antagonist with recombinant LH. LH activity supplementation improved the oocyte quality, implantation, and pregnancy outcomes in the recipients. In our study, we were also able to assess whether there were any endometrial effects of GnRH-antagonists, because all our patients underwent embryo transfer. We could not confirm the hypothesized decreased endometrial receptivity.

Animal and human studies have confirmed the presence of GnRH receptors in the endometrium [9, 10]. GnRH-antagonists have been shown to inhibit the synthesis of growth factors, to induce apoptosis, and also to decrease an already compromised uterine receptivity during ovarian stimulation with gonadotropins [11, 12]. However, in a randomized study comparing the outcomes of ovarian stimulation with pituitary down-regulation with GnRH-agonists versus GnRH-antagonist, Xavier et al. found comparable endometrial thickness on the day of hCG administration (and comparable serum estradiol levels), and pregnancy outcomes [13]. In our study, as well, we were not able to find the use of GnRH-antagonist a predictor of implantation failure. Alternatively, increasing age was a strong determinant of decreasing implantation. In another study by Kolibianakis et al., increasing the FSH dose alone at the time of GnRH-antagonist initiation was not associated with improved pregnancy outcomes [14]. In addition, this approach seemed not to improve endometrial

maturity as assessed by histologic examination. In light of these last results, we believe our findings were mainly due to LH activity, rather than FSH supplementation.

Although the possibility of an increased miscarriage rate as a consequence of endometrial dysfunction has been hypothesized, the impact of GnRH-antagonists on endometrial development has been evaluated only indirectly in human studies [7, 15]. In our study, once a pregnancy started, the risk of early loss, both in the form of a biochemical pregnancy or a miscarriage, was mainly related to the thickness of endometrium. Yet again, endometrial thickness was not related to the duration of GnRH-antagonist administration, or to a decreased implantation rate. This suggests that the hypothesized negative effect of GnRH-antagonists on endometrial receptivity, if any, would most probably manifest at a functional level, and it is not perceptible by the sonographic dimensions.

The reported negative effect of GnRH-antagonist administration on oocyte and embryo quality was not confirmed in this study. Peak serum estradiol and total dose of gonadotropins were the only positive predictors found by our regression model. We hypothesize that the additional FSH and, mainly, LH administration during the conventional ovarian stimulation, as confirmed in Acevedo et al. study, corrected the drug’s adverse effects [5]. The lack of a control group with no LH/FSH addition, however, underscores our findings.

In conclusion, our study could not confirm the reported detrimental effect of GnRH-antagonists on oocyte quality or endometrial receptivity. Oocyte quality depended mainly on the amount of gonadotropin used and the peak serum estradiol; implantation depended mainly on the patient’s age; and early loss depended on the thickness of the endometrial stripe. We believe that the dose increase of FSH and mainly LH activity when the antagonist is started, may have corrected the previously reported adverse effects.

References

1. Fluker M, Grifo J, Leader A, Levy M, Meldrum D, Muasher SJ, Rinehart J, Rosenwaks Z, Scott RT, Schoolcraft W, Shapiro DB, North American Ganirelix Study Group. Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. *Fertil Steril* 2001; 75:38–45.
2. Borm G, Mannaerts B. Treatment with the gonadotropin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. The European Orgalutran Study Group. *Hum Reprod* 2000;15:1490–8.
3. Lindheim SR, Morales AJ. GnRH antagonists followed by a decline in serum estradiol results in adverse outcomes in donor oocyte cycles. *Hum Reprod* 2003;18:2048–51.

4. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotropin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2006;3:CD001750.
5. Acevedo B, Sanchez M, Gomez JL, Cuadros J, Ricciarelli E, Hernandez ER. Luteinizing hormone supplementation increases pregnancy rates in gonadotropin-releasing hormone antagonist donor cycles. *Fertil Steril* 2004;82:343–7.
6. Cedrin-Durnerin I, Grange-Dujardin D, Laffy A, Parneix I, Massin N, Galey J, Theron L, Wolf JP, Conord C, Clement P, Jayot S, Hugues JN. Recombinant human LH supplementation during GnRH antagonist administration in IVF/ICSI cycles: a prospective randomized study. *Hum Reprod* 2004;19:1979–84.
7. Hernandez ER. Embryo implantation and GnRH-antagonists. *Hum Reprod* 2000;15:1211–6.
8. Duijkers IJ, Klipping C, Willemsen WN, Krone D, Schneider E, Niebch G, Hermann R. Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotropin-releasing hormone antagonist cetrorelix in healthy female volunteers. *Hum Reprod* 1998;13:2392–8.
9. Murdoch WJ. Immunolocalization of a gonadotropin-releasing hormone receptor site in murine endometrium that mediates apoptosis. *Cell Tissue Res* 1995;282:527–9.
10. Raga F, Casañ EM, Kruessel JS, Wen Y, Huang HY, Nezhat C, Polan ML. Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. *Biol Reprod* 1998;59:661–9.
11. Grundker C, Schlotawa L, Viereck V, Eicke N, Horst A, Kairies B, Emons G. Antiproliferative effects of the GnRH antagonist cetrorelix and of GnRH-II on human endometrial and ovarian cancer cells are not mediated through the GnRH type I receptor. *Eur J Endocrinol* 2004;151:141–9.
12. Ruan HC, Zhu XH, Luo Q, Liu AX, Qian YL, Zhou CY, Jin F, Huang HF, Sheng JZ. Ovarian stimulation with GnRH agonist, but not GnRH antagonist, partially restores the expression of endometrial integrin beta3 and leukaemia-inhibitory factor and improves uterine receptivity in mice. *Hum Reprod* 2006;21:2521–9.
13. Xavier P, Gamboa C, Calejo L, Silva J, Stevenson D, Nunes A, Martinez-de-Oliveira J. A randomised study of GnRH antagonist (cetrorelix) versus agonist (busereline) for controlled ovarian stimulation: effect on safety and efficacy. *Eur J Obstet Gynecol Reprod Biol* 2005;120:185–9.
14. Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smitz J, Van Steirteghem A, Devroey P. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. *Fertil Steril* 2002;78:1025–9.
15. Papanikolaou EG, Camus M, Fatemi HM, Tournaye H, Verheyen G, Van Steirteghem A, Devroey P. Early pregnancy loss is significantly higher after day 3 single embryo transfer than after day 5 single blastocyst transfer in GnRH antagonist stimulated IVF cycles. *Reprod Biomed Online* 2006;12:60–5.