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ARTICLE

No association between endogenous LH and pregnancy in a GnRH antagonist protocol: part II, recombinant FSH

George Griesinger [a,*], Daniel B Shapiro [b], Efstratios M Kolibianakis [c], Han Witjes [d], Bernadette M Mannaerts [e]

[a] *Department of Obstetrics and Gynaecology, University Clinic of Schleswig-Holstein, Campus Luebeck, Luebeck, Germany;* [b] *Reproductive Biology Associates, Atlanta, GA, USA;* [c] *Unit for Human Reproduction, Medical School, Aristotle University of Thessaloniki, Papagerogiou General Hospital, Thessaloniki, Greece;* [d] *Biostatistics and Research Decision Sciences, Women's Health and Endocrine, MSD, Oss, The Netherlands;* [e] *Global Clinical Research, Women's Health and Endocrine, Merck Research Laboratories, MSD, The Netherlands*

[*] Corresponding author. *E-mail address:* georg.griesinger@frauenklinik.uni-luebeck.de (G Griesinger).

Georg Griesinger is a professor for reproductive medicine at the Department of Obstetrics and Gynecology, University Clinic of Schleswig–Holstein, Luebeck, Germany. He was trained at the University of Vienna, where he worked in trophoblast research for his MD thesis at the Department of Obstetrics, and then did postdoctoral studies at University College London, where he obtained an MSc in prenatal genetics and fetal medicine. His PhD was on ovarian stimulation for IVF using gonadotrophin-releasing hormone antagonists. His research interests include the endocrinology of ovarian stimulation, evidence-based medicine in reproductive health care and epidemiological studies in IVF.

Abstract The association between endogenous LH concentrations during ovarian stimulation in a gonadotrophin-releasing hormone (GnRH) antagonist protocol and pregnancy likelihood was examined in a large combined analysis of individualized patient data obtained after treatment with recombinant FSH and a GnRH antagonist prior to IVF/intracytoplasmic sperm injection. Data from 1764 patients from six randomized controlled trials were pooled for retrospective analysis. Ongoing pregnancy and miscarriage rates for patients stratified by LH percentiles were assessed. Patients in the lowest LH quartile (<P25) were younger with a higher predicted ovarian reserve and response compared with patients in the highest quartile (>P75). With adjustment for identified predictive factors of pregnancy, estimated odds ratios (95% confidence interval) for ongoing pregnancy for LH categories <P25 versus ≥P25, >P75 versus ≤P75 and <P25 versus >P75 were 0.96 (0.75–1.22), 1.13 (0.88–1.45) and 0.89 (0.66–1.21) on stimulation day 8, and 0.96 (0.76–1.21), 1.03 (0.82–1.30) and 0.95 (0.72–1.26) on the day of human chorionic gonadotrophin, respectively. No significant differences in pregnancy or miscarriage rates between the LH categories were observed. Endogenous LH concentrations have no association with the likelihood of ongoing pregnancy in women undergoing ovarian stimulation using a recombinant FSH/GnRH antagonist protocol.

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KEYWORDS: combined analysis, endogenous LH, GnRH antagonist, pregnancy rates

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Introduction

The association between endogenous LH concentrations and clinical outcome in a recombinant FSH (rFSH) gonadotrophin-releasing hormone (GnRH) antagonist protocol has not been studied extensively but should be understood before advocating clinical management decisions based on endogenous LH concentrations.

The first studies on the relationship between low endogenous LH concentrations and clinical outcome in GnRH antagonist protocols were published in 2004, by Merviel et al. (2004), who found no impact of low endogenous LH (≤0.5 IU/l) on clinical pregnancies in 270 patients following ovarian stimulation for IVF, and by Kolibianakis et al. (2004), who reported that profound LH suppression (≤0.5 IU/l) on stimulation day 8 in a study of 116 women was associated with a higher chance of achieving an ongoing pregnancy.

Previous dose-finding studies have indicated that high doses of GnRH antagonists may induce very profound LH suppression and reduce the probability of clinical pregnancy (Huirne et al., 2005; The Ganirelix Dose-finding Study Group, 1998). However, low serum LH concentrations, defined by percentile analysis in 110 patients treated with 0.25 mg GnRH antagonist, were not shown to be associated with the probability of pregnancy (Bosch et al., 2005).

A recent publication (Doody et al., 2010) reported no association between endogenous LH concentrations measured on stimulation days 1, 5 or 8 and ongoing pregnancy rates in 750 patients treated with daily rFSH and 0.25 mg ganirelix in the Engage trial (Devroey et al., 2009). The analysis of this trial was then extended with a study of endogenous LH concentrations measured on stimulation day 8 and on the day of human chorionic gonadotrophin (HCG) administration, and the probability of pregnancy in both the rFSH and corifollitropin alfa treatment arms of the study (approximately 750 patients in each treatment arm) (Doody et al., 2011, part I of this study). In this study, endogenous LH concentrations ranging between <0.6 IU/l and 5 IU/l were not associated with the chance of ongoing pregnancy, whereas serum progesterone concentration on stimulation day 8 and ovarian response (follicles on stimulation day 8/oocytes) appeared to be significant predictors of ongoing pregnancy. Moreover, this analysis demonstrated that patients with lower serum LH concentrations tend to be younger, with a higher ovarian reserve and a higher ovarian response than patients with higher LH concentrations. In the study by Kolibianakis et al. (2004), patients with lower serum LH concentrations during stimulation had a higher chance of pregnancy but were also slightly younger and had statistically significantly lower endogenous FSH concentrations on stimulation day 1.

The present study examined the association of endogenous LH concentrations during the follicular phase with ongoing pregnancy rates in large data sets derived from six randomized trials with a total of 1764 patients treated with rFSH in a GnRH antagonist (ganirelix) protocol prior to IVF or intracytoplasmic sperm injection (ICSI). Identified predictors from the rFSH arm of the Engage trial (Doody et al., 2011, part I) were included as covariates in a combined analysis of the six trials using individual patient data stratified by LH concentrations determined within each trial by a central laboratory.

Materials and methods

Ongoing pregnancy rates and miscarriage rates relative to endogenous serum LH concentrations during ovarian stimulation were assessed from the following six trials, all of which included a GnRH antagonist (ganirelix) treatment arm with a daily dosage of 0.25 mg: (i) Engage (Devroey et al., 2009); (ii) Ensure (Corifollitropin alfa Ensure Study Group, 2010); (iii) Xpect (NCT identifier NCT00778999, Nyboe Andersen et al., in press); (iv) Ganirelix EU (The European Orgalutran Study Group, 2000); (v) Ganirelix ME (The European and Middle East Orgalutran Study Group, 2001); and (vi) Ganirelix NA (The North American Ganirelix Study Group, 2001). Patients were normogonadotrophic women with an indication for ovarian stimulation prior to IVF or ICSI. In all six trials, only data from the rFSH/ganirelix arms were used for the current analyses.

Engage trial (rFSH arm only)

Women aged 18–36 years with bodyweight from >60 kg to ≤90 kg received daily 200 IU rFSH (Puregon/Follistim pen; Organon, The Netherlands) up to and including the day of HCG administration. From stimulation day 8, the dose of rFSH was adjusted if necessary, according to the ovarian response. The GnRH antagonist ganirelix (0.25 mg, Orgalutran/ganirelix acetate injection, Organon) was administered once daily s.c. starting on stimulation day 5 upto and including the day of HCG injection. Urinary HCG (10,000 IU or 5000 IU) was administered i.m. to induce final oocyte maturation (Devroey et al., 2009).

Ensure trial (rFSH arm only)

Women aged 18–36 years with bodyweight ≤60 kg received daily 150 IU rFSH (Puregon/Follistim pen) upto and including the day of HCG administration. From stimulation day 8, the dose of rFSH was adjusted if necessary, according to the ovarian response. The GnRH antagonist ganirelix (0.25 mg) was administered once daily s.c. starting on stimulation day 5 upto and including the day of HCG injection. Urinary HCG (10,000 IU) was administered i.m. to induce final oocyte maturation (Corifollitropin alfa Ensure Study Group, 2010).

Xpect trial (excluding the oral contraception pretreatment arm)

Women aged 18–39 years with body mass index (BMI) ≤32 kg/m^2 received daily 200 IU rFSH (Puregon/Follistim pen) upto and including the day of HCG administration, with dose adjustment as necessary after stimulation day 6. On stimulation day 5, the GnRH antagonist ganirelix (0.25 mg) was administered daily s.c. upto and including the day of HCG administration. To induce final oocyte maturation, 5000–10,000 IU HCG was administered (NCT identifier NCT00778999; Nyboe Andersen et al., in press).

European ganirelix trial (ganirelix arm only)

Women aged 18–39 years with BMI 18–29 kg/m^2 received daily 150 IU rFSH (Puregon/Follistim pen) from stimulation day 1 upto the day of HCG, with dose adjustment as necessary after stimulation day 6. From day 6 of rFSH treatment, the GnRH antagonist ganirelix (0.25 mg) was administered daily s.c. upto and including the day of HCG administration. Urinary HCG (10,000 IU, Pregnyl; Organon) was administered to induce final oocyte maturation (The European Orgalutran Study Group, 2000).

European and Middle East ganirelix trial (ganirelix arm only)

Women aged 18–39 years with BMI 18–29 kg/m^2 received daily 150 IU rFSH (Puregon/Follistim pen) from stimulation day 1 upto the day of HCG, with dose adjustment after day 6 depending on the ovarian response as monitored by ultrasound scan. From day 6 of rFSH treatment, the GnRH antagonist ganirelix (0.25 mg) was administered daily s.c. upto and including the day of HCG administration. Urinary HCG (10,000 IU, Pregnyl) was administered to induce final oocyte maturation (The European and Middle East Orgalutran Study Group, 2001).

North American ganirelix trial (ganirelix arm only)

Women aged 18–39 years with BMI 18–29 kg/m^2 received daily 225 IU rFSH (Puregon/Follistim pen) from stimulation day 1 upto the day of HCG, with dose adjustment after day 6 depending on the ovarian response. From day 6 of rFSH treatment, the GnRH antagonist ganirelix (0.25 mg) was administered daily s.c. upto and including the day of HCG administration. Urinary HCG (10,000 IU, Pregnyl) was administered to induce final oocyte maturation (The North American Ganirelix Study Group, 2001).

General trial criteria

In all six trials, patients with an irregular menstrual cycle were excluded. Stimulation was always started on days 2 or 3 of menses and final oocyte maturation by HCG was triggered when at least three follicles $\geq$17 mm were observed by ultrasound scan. Patients had a transvaginal ultrasound-guided oocyte retrieval 34–36 h after 10,000 IU urinary HCG (Pregnyl) administration, followed by either IVF or ICSI.

In the Engage, Ensure and Xpect trials, luteal phase support with progesterone (at least 600 mg/day vaginally or at least 50 mg/day i.m.) was started on the day of oocyte retrieval and continued for at least 6 weeks. In the other three trials, luteal support was given according to the clinic's routine practice. Ongoing pregnancy rates were calculated based on the presence of at least one fetus with heart activity at least 10 weeks after embryo transfer. The miscarriage rate was calculated for subjects with a clinical pregnancy defined as the presence of at least one gestational sac.

Hormone assessments were made in blood samples drawn in the morning just prior to GnRH antagonist and gonadotrophin injections and the serum immediately stored at −20°C until analysis.

Validated immunoassays were performed to measure serum concentrations of FSH, LH, oestradiol and progesterone at stimulation days 1, 5 and 8 and day of HCG. In all trials except for the Ganirelix NA trial, these hormones were determined at one central laboratory (Waltrop, Germany) using a time-resolved fluoroimmunoassay (AutoDelfia immunofluorometric assay; PerkinElmer Life and Analytical Sciences, Brussels, Belgium). In the Ganirelix NA trial, serum LH was measured by the Immulite 1000 LH assay (DPC, Los Angeles, CA) and oestradiol and progesterone at a central laboratory (Quest Diagnostics, USA).

Statistical analysis

All analyses included the intent-to-treat (ITT) groups comprising subjects randomized to rFSH treatment who started stimulation. Per trial, patients were stratified according to the 25th and 75th percentiles (P25 and P75) of serum LH concentrations, resulting in three groups of patients <P25 (low LH), P25–P75 (medium LH) and >P75 (high LH). Patients without LH measurements were excluded from these groups.

Overall differences in baseline characteristics, stimulation characteristics and ovarian response between low (<P25), medium (P25–P75) and high (>P75) serum LH concentrations on stimulation day 8 were presented and tested for statistically significant differences between LH categories using either analysis of variance (ANOVA) for comparing means or the Kruskal–Wallis test for comparing medians. A *P*-value $\leq$0.05 was considered statistically significant. No multiplicity correction was applied (*P*-values were not corrected for multiple testing in order to control the overall type I error rate of 0.05). The <P25, P25–P75 and >P75 groups of patients of the separate trials on stimulation day 8 were pooled per LH category for this purpose.

Differences in ongoing pregnancy rates between low (<P25), medium (P25–P75) and high (>P75) LH concentrations were estimated per trial and overall (combined). The individual patient data of the six trials were fitted to a logistic regression model using PROC GENMOD in SAS version 9.1 (SAS Institute, Cary, NC, USA). The ongoing pregnancy rate was modeled as a function of trial (six-level class variable), LH category (three-level class variable, i.e. <P25, P25–P75 and >P75) and several identified predictive factors of ongoing pregnancy. The predictive factors were identified using the data of the rFSH arm from the Engage trial (Doody et al., 2011, part I). The miscarriage rate was modeled as a function of trial and LH category for estimation of the differences in miscarriage rate between the <P25, P25–P75 and >P75 groups of patients per trial and overall. Separate models of ongoing pregnancy rate and miscarriage rate were obtained for the LH categories on stimulation day 8 and day of HCG, respectively.

Trial by LH interaction was first added to the model to determine the heterogeneity of the LH effect across the six trials. If the *P*-value for interaction was >0.10, then the effect across the six trials was considered homogeneous and the model without interaction was applied to provide estimates per trial and overall. If the *P*-value for heterogeneity was <0.10, then estimates are not provided.

Table 1 Mean baseline characteristics of all patients included in the combined LH analysis presented by LH on stimulation day 8.

Variable	*LH percentile category*			*P-value*
	<P25 (n = 436)	*P25–P75 (n = 891)*	*>P75 (n = 437)*	
Age (years)	31.2 ± 3.5	31.6 ± 3.6	31.8 ± 3.7	0.02[a]
Body mass index (kg/m^2)	23.3 ± 3.0	23.7 ± 3.0	23.9 ± 3.1	0.01[a]
Bodyweight (kg)	64.4 ± 9.2	64.7 ± 9.0	65.1 ± 9.3	NS[a]
Serum LH on day 1	4.1 (2.0, 7.2)	4.6 (2.4, 7.9)	5.0 (2.7, 9.4)	<0.01[b]
Serum FSH on day 1	6.1 (3.5, 10.0)	6.4 (4.2, 10.5)	6.7 (4.5, 11.6)	<0.01[b]

Values are mean ± standard deviation or median (P5, P95). NS = not statistically significant; <P25 = patients below the 25th LH percentile; P25–P75 = patients between the 25th and 75th LH percentiles; >P75 = patients above the 75th LH percentile.
[a]Analysis of variance.
[b]Kruskal–Wallis test.

In case of homogeneity ($P > 0.10$), *P*-values of the overall LH effect based on the likelihood ratio test were provided and maximum likelihood estimates of odds ratios (OR) and associated two-sided 95% confidence intervals (CI) of <P25 versus ≥P25 (low versus not low LH), >P75 versus ≤P75 (high versus not high LH) and <P25 versus >P75 (low versus high LH) per trial and overall were presented. For the ongoing pregnancy-rate model, the *P*-values and estimated OR and CI with and without adjustment for the predictive factors are presented.

Results

Patient population

In total, the LH analysis included 1764 patients. Age, BMI, bodyweight and serum FSH and LH concentrations on stimulation day 1 per LH percentile category on stimulation day 8 (<P25, P25–P75 and >P75) are presented in **Table 1**. Patients with higher serum LH concentrations on stimulation day 8 were older and also had slightly higher baseline FSH and LH concentrations than patients with lower serum LH concentrations at stimulation day 8.

Serum LH concentrations on stimulation day 8

Serum LH concentrations per percentile (P5, P25, P50, P75 and P95) in each trial are presented in **Table 2**. On stimulation day 8, the P50 (median) value ranged from 1.32 IU/l to 2.10 IU/l between trials. The P25 values varied between 0.78 IU/l and 1.30 IU/l and the P75 values ranged from 1.97 IU/l to 3.25 IU/l.

Table 2 LH concentrations in LH percentile categories on stimulation day 8 by trial.

Trial	*LH percentiles*				
	P5	*P25*	*P50*	*P75*	*P95*
Engage	<0.6	0.91	1.57	2.66	5.27
Ensure	<0.6	0.81	1.60	2.71	5.52
Xpect	<0.6	1.04	1.79	3.25	6.16
Ganirelix EU	<0.6	0.86	1.32	2.23	4.94
Ganirelix ME	<0.6	0.78	1.32	1.97	4.63
Ganirelix NA	0.50	1.30	2.10	2.90	4.90

Stimulation characteristics and ovarian response per LH category

The duration of stimulation, the total dose of rFSH and endocrine parameters per LH percentile category are presented in **Table 3**. Patients with lower LH concentrations required 0.5 days longer stimulation and on average 100 IU rFSH more than patients with higher LH concentrations to reach the same criteria for HCG administration.

On stimulation day 8 and day of HCG, patients with higher LH concentrations had a lower number of follicles ≥11 mm whereas their serum oestradiol and progesterone concentrations were higher compared with patients with lower LH concentrations. In line with the lower number of follicles, fewer oocytes were recovered in patients with higher LH concentrations: the estimated difference was 1.9 oocytes compared with patients with lower LH concentrations.

Ongoing pregnancy rates per LH category and trial

Per trial, the ongoing pregnancy rates in the <P25, P25–P75 and >P75 groups of patients on stimulation day 8 and day of HCG are shown in **Table 4**.

Estimated LH effects on ongoing pregnancy

Neither the overall LH effect nor the trial by LH interaction effect (heterogeneity) were statistically significant based on the LH categories on stimulation day 8 or day of HCG, with or without adjustment of the predictive factors (**Table 5**).

The estimated OR for ongoing pregnancy rates by LH category on stimulation day 8 and day of HCG administration are given in **Table 6**. In patients with low LH concentrations, the estimated overall OR for ongoing pregnancy rate of <P25 versus ≥P25 on stimulation day 8 was 0.96 (95% CI 0.75–1.22), when adjusted for trial, age, number of oocytes retrieved and serum progesterone concentration

Table 3 Stimulation characteristics and ovarian response of all patients included in the combined LH analysis presented by LH on stimulation day 8.

Characteristic/response	*LH percentile category*			P-*value*
	<P25 (n = 436)	*P25–P75 (n = 891)*	*>P75 (n = 437)*	
Duration of stimulation (days)	10.0 ± 1.7	9.8 ± 1.7	9.5 ± 1.5	<0.01
Total dose of rFSH (IU)	1759 ± 522	1729 ± 469	1652 ± 457	<0.01
Follicles on day 8				
≥11 mm	10.4 ± 5.8	10.0 ± 5.6	9.5 ± 5.2	0.02
≥15 mm	4.9 ± 4.5	5.1 ± 4.0	4.9 ± 3.9	NS
≥17 mm	2.0 ± 2.6	2.4 ± 2.7	2.6 ± 2.9	0.01
Hormones on day 8				
Oestradiol (pmol/l)	2007 (573, 7670)	2936 (635, 8973)	3854 (998, 9982)	<0.01
Progesterone (nmol/l)	1.7 (<1, 4.2)	1.9 (<1, 4.3)	2.3 (1.1, 4.7)	<0.01
Follicles on day of human chorionic gonadotrophin				
≥11 mm	13.1 ± 6.3	12.2 ± 6.1	11.0 ± 5.8	<0.01
≥15 mm	8.8 ± 4.3	8.2 ± 4.1	7.2 ± 3.9	<0.01
≥17 mm	5.6 ± 3.1	5.2 ± 2.8	4.9 ± 2.7	<0.01
Hormones on day of human chorionic gonadotrophin				
Oestradiol (pmol/l)	3622 (1240, 9564)	4991 (1809, 11,671)	5975 (2019, 13,212)	<0.01
Progesterone (nmol/l)	2.3 (1.2, 5.8)	2.6 (1.2, 5.3)	2.9 (1.3, 5.9)	<0.01
Oocytes	11.9 ± 6.8	11.1 ± 6.7	10.0 ± 6.1	<0.01

Values are mean ± standard deviation or median (P5, P95). rFSH, recombinant FSH; NS = not statistically significant.

Table 4 Ongoing pregnancy rate per started cycle, by trial and LH on stimulation day 8 and the day of HCG.

Trial	*rFSH start dose (IU)*	*Ongoing pregnancy rate by LH category*		
		<P25	*P25–P75*	*>P75*
Stimulation day 8				
Engage	200	60/169 (35.5)	125/340 (36.8)	65/169 (38.5)
Ensure	150	12/30 (40.0)	20/61 (32.8)	10/30 (33.3)
Xpect	200	17/42 (40.5)	33/87 (37.9)	13/41 (31.7)
Ganirelix EU	150	20/105 (19.0)	44/210 (21.0)	23/105 (21.9)
Ganirelix ME	150	19/49 (38.8)	29/103 (28.2)	13/50 (26.0)
Ganirelix NA	225	15/41 (36.6)	25/90 (27.8)	14/42 (33.3)
Day of human chorionic gonadotrophin				
Engage	200	63/175 (36.0)	132/352 (37.5)	74/174 (42.5)
Ensure	150	10/31 (32.3)	25/65 (38.5)	9/30 (30.0)
Xpect	200	18/45 (40.0)	37/92 (40.2)	11/45 (24.4)
Ganirelix EU	150	21/112 (18.8)	55/224 (24.6)	19/112 (17.0)
Ganirelix ME	150	20/53 (37.7)	29/109 (26.6)	21/53 (39.6)
Ganirelix NA	225	16/46 (34.8)	28/96 (29.2)	17/45 (37.8)

Values are *n*/total (%) unless otherwise stated. rFSH, recombinant FSH.

on stimulation day 8 (**Figure 1**). The estimated overall OR for ongoing pregnancy of <P25 versus ≥P25 on the day of HCG administration was 0.96 (95% CI 0.76–1.21), when adjusted for trial and age. For patients with high LH concentrations, the estimated overall OR for ongoing pregnancy rate of >P75 versus ≤P75 on stimulation day 8 was 1.13 (95% CI 0.88–1.45) when adjusted for trial, age, number of oocytes retrieved and serum progesterone concentration on stimulation day 8. The estimated overall OR for ongoing pregnancy rate of >P75 versus ≤P75 on the day of HCG administration was 1.03 (95% CI 0.82–1.30) when adjusted for trial and age.

In summary, neither low nor high endogenous LH concentrations on stimulation day 8 or day of HCG showed a

Table 5 Logistic regression model for ongoing pregnancy by LH and heterogeneity (trial by LH interaction) per day.

Model	*P-value*	
	LH effect	*Heterogeneity*
Stimulation day 8		
Adjusted for trial	0.79	0.91
Adjusted for trial, age, no. of oocytes retrieved and progesterone concentration on day 8	0.59	0.94
Day of human chorionic gonadotrophin		
Adjusted for trial	0.96	0.17
Adjusted for trial and age	0.94	0.16

Table 6 Logistic regression model for ongoing pregnancy in LH percentile categories per started cycle, per trial and overall on stimulation day 8 and day of human chorionic gonadotrophin.

Trial	*<P25 versus ≥P25*	*>P75 versus ≤P75*	*<P25 versus >P75*
Stimulation day 8			
Engage	0.91 (0.63–1.32)	1.10 (0.77–1.59)	0.88 (0.57–1.37)
Ensure	1.35 (0.57–3.21)	0.88 (0.36–2.12)	1.33 (0.46–3.82)
Xpect	1.28 (0.61–2.65)	0.72 (0.34–1.54)	1.46 (0.59–3.61)
Ganirelix EU	0.86 (0.49–1.52)	1.12 (0.65–1.94)	0.84 (0.43–1.64)
Ganirelix ME	1.71 (0.86–3.40)	0.71 (0.34–1.46)	1.80 (0.77–4.23)
Ganirelix NA	1.32 (0.62–2.78)	1.06 (0.50–2.25)	1.15 (0.47–2.85)
Overall	1.07 (0.85–1.36)[a]	0.99 (0.78–1.25)[a]	1.06 (0.79–1.41)[a]
	0.96 (0.75–1.22)[b]	1.13 (0.88–1.45)[b]	0.89 (0.66–1.21)[b]
Day of human chorionic gonadotrophin			
Engage	0.84 (0.59–1.21)	1.27 (0.89–1.82)	0.76 (0.49–1.17)
Ensure	0.92 (0.38–2.23)	0.79 (0.32–1.94)	1.11 (0.38–3.29)
Xpect	1.43 (0.70–2.93)	0.48 (0.22–1.04)	2.06 (0.83–5.09)
Ganirelix EU	0.89 (0.51–1.56)	0.75 (0.42–1.32)	1.13 (0.57–2.24)
Ganirelix ME	1.24 (0.65–2.39)	1.40 (0.73–2.69)	0.92 (0.42–2.02)
Ganirelix NA	1.07 (0.52–2.17)	1.30 (0.64–2.63)	0.88 (0.37–2.07)
Overall	0.97 (0.77–1.22)[a]	1.03 (0.82–1.30)[a]	0.96 (0.73–1.27)[a]
	0.96 (0.76–1.21)[c]	1.03 (0.82–1.30)[c]	0.95 (0.72–1.26)[c]

Values are estimated OR (95% confidence interval).
[a]Adjusted for trial.
[b]Adjusted for trial, age, number of oocytes retrieved and serum progesterone concentration on day 8.
[c]Adjusted for trial and age.

significant association with ongoing pregnancy likelihood. The estimated OR were not statistically significantly different from 1.0.

Miscarriage rates within trials according to LH category

Miscarriage rates per started cycle within each trial in the <P25, P25–P75 and >P75 groups of patients on stimulation day 8 and day of HCG are shown in **Table 7**.

Estimated LH effects on miscarriage

Trial by LH interaction (heterogeneity) on day of HCG was statistically significant ($P = 0.05$). Therefore, differences in miscarriage rates among the three LH categories on day of HCG could not be estimated.

Trial by LH interaction (heterogeneity) on stimulation day 8 was borderline significant. The overall LH effect on stimulation day 8 was not statistically significant. In patients with low LH concentrations, the estimated overall OR for miscarriage rate of <P25 versus ≥P25 on stimulation day 8 (adjusted for trial) was 1.07 (95% CI 0.59–1.94). For patients with high LH concentrations, the estimated overall OR for miscarriage rate of >P75 versus ≤P75 on stimulation day 8 (adjusted for trial) was 0.87 (95% CI 0.47–1.60).

In summary, neither low nor high endogenous LH concentrations on stimulation day 8 showed an association with the likelihood of miscarriage. The estimated OR were not statistically significantly different from 1.0.

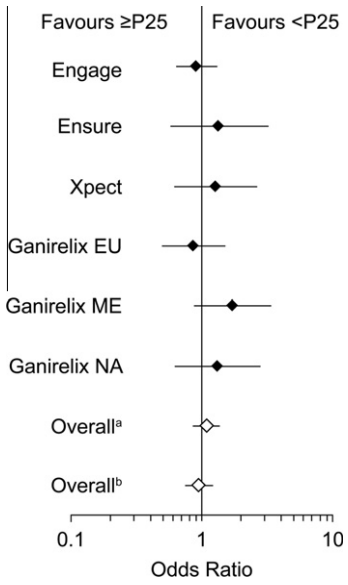

Figure 1 Estimated odds ratios (per trial and overall) of patients in the LH percentile group <P25 versus patients in ≥P25 group for ongoing pregnancy rate per started cycle on stimulation day 8. [a]Adjusted for trial.
[b] Adjusted for trial, age, number of oocytes retrieved and serum progesterone concentration on stimulation day 8.

Discussion

Neither low nor high endogenous LH concentrations were associated with the likelihood of pregnancy in a pooled analysis of individual LH data of 1764 patients from six clinical trials that used rFSH for ovarian stimulation in a GnRH antagonist protocol. In addition to the lack of effect of endogenous LH concentrations on pregnancy rates, the current analyses showed that neither low nor high LH concentrations impacted miscarriage rates.

The validity of the pooled analyses was shown by the applied heterogeneity tests. The LH effects on pregnancy achievement across the six trials were demonstrated to be homogeneous, while the LH effects on miscarriage rates showed some variation across the trials.

The current analyses have the advantage that the LH assessments were carried out for all patients just prior to GnRH antagonist injection (Griesinger et al., 2006) and were analysed by a central laboratory allowing a more consistent analysis of the impact of endogenous LH on clinical outcome. The disadvantage of measuring serum LH concentrations 24 h after GnRH antagonist administration, just prior to the next antagonist injection, is that the absolute values are much higher than the LH nadir, which is reached after 4 h (Oberyé et al., 1999).

It should be noted that the included patient population had normal serum FSH and LH concentrations in the early follicular phase, were relatively young (up to age 39 years) and consisted of (potentially) normal-responder patients. Within this population, the current pooled LH analysis confirmed the observation that patients with lower pre-ovulatory LH concentrations tend to be younger with a higher ovarian reserve, as reflected by their lower baseline FSH and LH at stimulation day 1, resulting in a higher ovarian response than in patients with higher pre-ovulatory LH concentrations (Doody et al., 2011, part I). None of the patients included in this pooled LH analysis were pre-treated with oral contraceptives or any other hormonal preparation within 1 month prior to randomization. The current pooled LH analysis was performed on stimulation day 8 and included all subjects who started stimulation and who provided a blood sample for hormone analysis on the specified day. Importantly, it included all subjects regardless of whether they discontinued prior to HCG administration or before embryo transfer.

Table 7 Miscarriage rate per started cycle for subjects with a clinical pregnancy presented by trial and LH category on stimulation day 8 and day of human chorionic gonadotrophin.

Trial	*rFSH start dose (IU)*	*Miscarriage rate by LH category*		
		<P25	*P25–P75*	*>P75*
Stimulation day 8				
Engage	200	5/169 (3.0)	11/340 (3.2)	4/169 (2.4)
Ensure	150	2/30 (6.7)	2/61 (3.3)	0/30 (0.0)
Xpect	200	6/42 (14.3)	4/87 (4.6)	1/41 (2.4)
Ganirelix EU	150	1/105 (1.0)	7/210 (3.3)	3/105 (2.9)
Ganirelix ME	150	0/49 (0.0)	4/103 (3.9)	4/50 (8.0)
Ganirelix NA	225	2/41 (4.9)	5/90 (5.6)	2/42 (4.8)
Day of human chorionic gonadotrophin				
Engage	200	6/175 (3.4)	9/352 (2.6)	5/174 (2.9)
Ensure	150	1/31 (3.2)	3/65 (4.6)	0/30 (0.0)
Xpect	200	7/45 (15.6)	6/92 (6.5)	1/45 (2.2)
Ganirelix EU	150	3/112 (2.7)	5/224 (2.2)	4/112 (3.6)
Ganirelix ME	150	0/53 (0.0)	8/109 (7.3)	0/53 (0.0)
Ganirelix NA	225	2/46 (4.3)	6/96 (6.3)	2/45 (4.4)

Values are *n*/total (%).

The predictive factors for ongoing pregnancy in addition to age that were applied for adjustment of the estimated OR were derived from a stepwise logistic regression analysis of the rFSH arm of the largest of the six included trials, the Engage trial (Doody et al., 2011, part I). Moreover, these factors (progesterone concentration and number of oocytes retrieved) and age have previously been described to impact pregnancy rates (Bosch et al., 2010; Broekmans et al., 2006).

An alternative approach to identify predictive factors for ongoing pregnancy would be to pool the data of the rFSH/ganirelix arms of the six trials. However, the applied model should take into account possible trial effects on each of the candidate predictive factors. These trial effects could be related to differences in the trial population, hormone assays and the treatment regimen. Accurate adjustment for trial effect in this particular application is difficult to justify and therefore this approach was not considered.

To date, the number of studies addressing the impact of endogenous LH concentrations in GnRH antagonist protocols is limited in contrast to various univariate analyses of the associations between mid-follicular concentrations of LH and pregnancy rates in long GnRH agonist protocols (Balasch et al., 2001; Cabrera et al., 2005; Esposito et al., 2001; Humaidan et al., 2002; Nakagawa et al., 2008; Westergaard et al., 2000), which have been reported with inconclusive results. Due to differences in the duration and extent of LH suppression in long GnRH agonist protocols, caution should be exerted when comparing pregnancy outcomes relative to LH concentrations from trials using different compounds, doses or route of administration of GnRH agonists.

Although the absolute concentrations of endogenous LH in a GnRH antagonist protocol do not affect clinical outcome during ovarian stimulation with rFSH, it may still be that addition of recombinant LH or HCG during stimulation positively affects clinical outcome. In a systematic review and meta-analysis to assess whether the addition of recombinant LH during ovarian stimulation increases live-birth rates, seven randomized clinical trials were identified, five with a long GnRH agonist protocol and two with a GnRH antagonist protocol (Kolibianakis et al., 2007). No significant difference in the probability of live birth was found for patients treated with or without recombinant LH supplementation (OR 0.92, 95% CI 0.65—1.31). This finding held in subgroup analyses that ordered the studies by dose of recombinant LH added, the type of analog used to inhibit premature LH surge, the time recombinant LH was added during the follicular phase and the age of patients analysed.

In the current investigation, the combined analyses of the individual patient data from six trials confirmed the findings of the analysis of the Engage trial (Doody et al., 2011, part I). Neither low nor high endogenous LH concentrations affected ongoing pregnancy rates, and in principle the amount of endogenous LH was sufficient to support rFSH during ovarian stimulation prior to IVF or ICSI. The validity of these findings at stimulation day 8 rather than day of HCG may be more relevant as the latter analysis excludes patients who were discontinued prior to HCG administration. Furthermore, from a clinical point of view, the results on stimulation day 8 are more relevant, since LH supplementation in those patients with low endogenous LH after GnRH antagonist initiation on stimulation days 5 or 6 of stimulation is still possible.

In conclusion, a GnRH antagonist treatment during ovarian stimulation results in variable amounts of endogenous circulating LH. Combined analyses of individual LH concentrations from 1764 patients from six trials who underwent ovarian stimulation with rFSH in a GnRH antagonist protocol show that ongoing pregnancy rates were not associated with the extent of LH suppression as measured on stimulation day 8 and day of HCG. This is the largest combined analysis to date to evaluate the association of endogenous LH concentrations with the likelihood of pregnancy in a GnRH antagonist protocol controlling for significant covariates shown to influence pregnancy rates. It provides robust evidence from prospective trials measuring serum LH in a central laboratory that endogenous LH concentrations have no association with the chance of ongoing pregnancy in normogonadotrophic women undergoing ovarian stimulation with rFSH. Thus, endogenous LH concentrations cannot serve as a rationale for adding LH activity to a GnRH antagonist ovarian stimulation protocol.

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