Article

Randomized controlled pilot trial of luteal phase recombinant FSH stimulation in poor responders

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Abstract

This study investigated whether luteal phase initiation of FSH supplementation would improve oocyte yield compared with follicular phase administration in women with poor ovarian response (POR). A two-arm, randomized, open-label pilot trial was performed at a university-based infertility centre. In nine of 18 infertile women with a history of POR in a previous cycle [<5 follicles on day of human chorionic gonadotrophin (HCG) administration; <5 oocytes retrieved; previous IVF cycle cancellation due to POR] FSH was administered during the mid-luteal phase of the preceding menstrual cycle. The primary outcome measure was the number of oocytes retrieved. Secondary endpoints included: follicles >10 mm and >16 mm and oestradiol concentration on the day of HCG administration, peak oestrogen concentration, pregnancy and live birth rates. All endpoints comparing luteal versus follicular stimulation were similar. In paired analysis of patients in the luteal arm compared with the prior cycle, there was a significant increase in days of stimulation ($P = 0.01$) and number of follicles >10 mm ($P = 0.02$) and >16 mm ($P = 0.02$) on day of HCG administration. IVF outcomes with luteal phase FSH compared with follicular phase FSH were similar. Luteal phase initiation of FSH is a safe and potential alternative protocol in poor responders.

Keywords: *FSH, GnRH antagonist, IVF, luteal phase, poor ovarian response, recombinant FSH*

Introduction

Poor ovarian response after ovarian stimulation for IVF is a challenge for the patient and clinician. Various criteria have been used to define poor response, including peak oestradiol concentration < 1000 pg/ml, fewer than five dominant follicles the day of human chorionic gonadotrophin (HCG) administration, fewer than five retrieved oocytes, at least one cancelled cycle due to poor response, and an increased dose requirement for exogenous FSH during IVF stimulation (Tartlazis *et al*., 2003). Although there is no universal definition, it is widely accepted that these patients develop fewer follicles, and subsequently have fewer retrieved oocytes and lower pregnancy rates following IVF.

A major goal in optimizing ovarian response involves the gonadotrophin rescue of a larger cohort of follicles during ovarian stimulation. Ovarian folliculogenesis is an ongoing process in which multiple follicles are in the process of development. The purpose of ovarian stimulation is to provide a high concentration of FSH to limit the number of oocytes that undergo atresia. Multiple regimens have been proposed to optimize ovarian response and pregnancy rates in these patients (Griesinger *et al*., 2006; Shanbhag *et al*., 2007; Sunkara *et al*., 2007). Few of these regimens have been compared directly in clinical trials.

Standard diminished ovarian reserve protocols initiate high doses of recombinant FSH (rFSH) in the follicular phase of the menstrual cycle in an attempt to augment early follicular maturation and minimize atresia. A major limitation of this type of protocol is the number of oocytes already recruited in the developing cohort (Rombauts *et al.*, 1998). Although the follicular phase is classically thought to be the phase of follicular growth and recruitment, it has been shown that, in a natural cycle, FSH first begins to rise during the preceding luteal phase, 12 days after the LH surge (Hall *et al.*, 1992). This mid-luteal rise of FSH in the preceding menstrual cycle suggests a potential role in the luteofollicular transition of follicular development.

This study reports a pilot randomized controlled trial to investigate whether an IVF protocol initiating rFSH in the luteal phase of women with a history of poor ovarian response would improve oocyte yield compared with a standard follicular phase protocol. In both regimens, gonadotrophin-releasing hormone (GnRH) antagonist was used to prevent premature LH surge and ovulation.

Materials and methods

The study was a two-arm, randomized, open-label pilot trial consisting of 18 patients at a university-based infertility program (Hospital of University of Pennsylvania). The 11-month enrolment period commenced in September 2005 and the last patient was randomized in July 2006. The patients were infertile women aged 20–42 years, planning to undergo IVF, with a history of poor response to IVF stimulation within the previous 12 months. Poor ovarian response in a prior cycle was defined as any of the following: <5 follicles on the day of HCG administration; <5 oocytes retrieved; or cancellation of a previous IVF cycle due to poor response to ovarian stimulation. Patients had to have both ovaries and at least 45 days had to have elapsed from their last IVF, ovarian stimulation or clomiphene citrate cycle (**Figure 1**). Exclusion criteria included significant systemic disease, regular cigarette smoking, undiagnosed vaginal bleeding or known allergy to gonadotrophin preparation. The patients were subject to post-randomization exclusion if their baseline day 3 FSH concentration exceeded 12.0 mIU/ml [Immulite assay (Diagnostic Products Corporation, Flanders, NJ); normal range follicular phase 2.8–11.3 mIU/ml] in two separate menstrual cycles, in accordance with the Centre's standard practice at the time of the study.

Once eligibility was confirmed, patients were randomized by a third party with opaque, sealed envelopes via random number computer-generated block randomization to begin recombinant FSH in the luteal phase or in the follicular phase. In the luteal phase regimen, patients were instructed to begin rFSH [Follistim AQ Cartridge (follitropin beta injection), Organon USA Inc., Roseland, NJ, USA] 9 days after spontaneous LH surge of the menstrual cycle preceding oocyte retrieval at a dose of 150 IU subcutaneously twice a day, and on day 1 of menses, the dose was increased to 300 IU twice a day in an attempt to mimic the natural pattern of the luteofollicular transition. Patients in the follicular arm began rFSH at the maximum dose (300 IU twice a day) on cycle day 1 or 2 of the oocyte retrieval cycle.

After randomization, all patients were scheduled to return to the office on cycle day 1 or 2 of their subsequent menses. At this baseline visit, blood tests (including oestradiol, FSH, LH and HCG) and transvaginal ultrasound examination of ovaries and uterus were performed. Ovulation was confirmed by LH measurement and visualization of the corpus luteum on ultrasound. Depending on treatment allocation, patients were instructed to start their medication that evening (follicular protocol) or to return 9 days after spontaneous LH surge (luteal protocol). On approximately day 23 (9 days after a spontaneous LH surge), patients in the luteal arm returned for repeat baseline blood tests (oestradiol, FSH, LH and HCG) and pelvic ultrasound. After pre-existing pregnancy was excluded, stimulation with recombinant FSH was initiated as described above.

In both arms of the study, follicular development during treatment was monitored according to standard practice with serial transvaginal ultrasound examinations and oestradiol concentrations. The remainder of ovarian stimulation was the same for both groups. GnRH antagonist (ganirelix acetate 250 µg/0.5 ml s.c. q.d., Organon USA Inc., Roseland, NJ, USA) was administered in conjunction with rFSH when the lead follicle was 12 mm. Triggering of final oocyte maturation with HCG (Pregnyl 10,000 units; Organon) was administered when at least one follicle with a mean diameter greater than or equal to 18 mm was present. Transvaginal ultrasound-guided oocyte retrieval, oocyte insemination and embryo transfer were performed according to standard practice in all patients. Luteal support with natural progesterone in oil (Abraxis, CA, USA; 50 mg/ml i.m.) was administered daily starting on the day of oocyte retrieval and, in patients who became pregnant, continued until 10 weeks' gestation. Given the difference in start date for each regimen, it was not possible to blind the subjects or clinical staff to protocol.

The primary endpoint in this pilot study was the number of oocytes retrieved. A priori sample size calculation determined that nine patients were needed to complete one treatment cycle in each arm. This sample size was determined using estimates from preliminary data on poor responders from clinical experience at the University of Pennsylvania. According to this data, the mean number of oocytes retrieved in poor responders was two with a standard deviation of one. To normalize the data for the purpose of the sample size calculation, the square root of these values was used. The calculation for a difference in means between two independent samples was used to determine sample size for this trial. A difference in two oocytes, or a 100% increase in oocyte yield, between the two regimens was considered a clinically significant difference in ovarian response. Other assumptions included an alpha of 0.05, power of 0.80, and a 1:1 ratio of treatment allocation between the groups.

Secondary outcomes included: number of follicles >10 and >16 mm and oestradiol concentration on the day of HCG administration, the peak oestradiol concentration, clinical pregnancy rate per transfer and delivery rate per transfer. Safety endpoints included ovarian torsion, ovarian hyperstimulation syndrome, enlarging cysts and serious adverse events.

Prior to statistical analysis, data were evaluated for normality. Given the lack of normality in this sample, non-parametric tests were used to compare the luteal and follicular protocols.

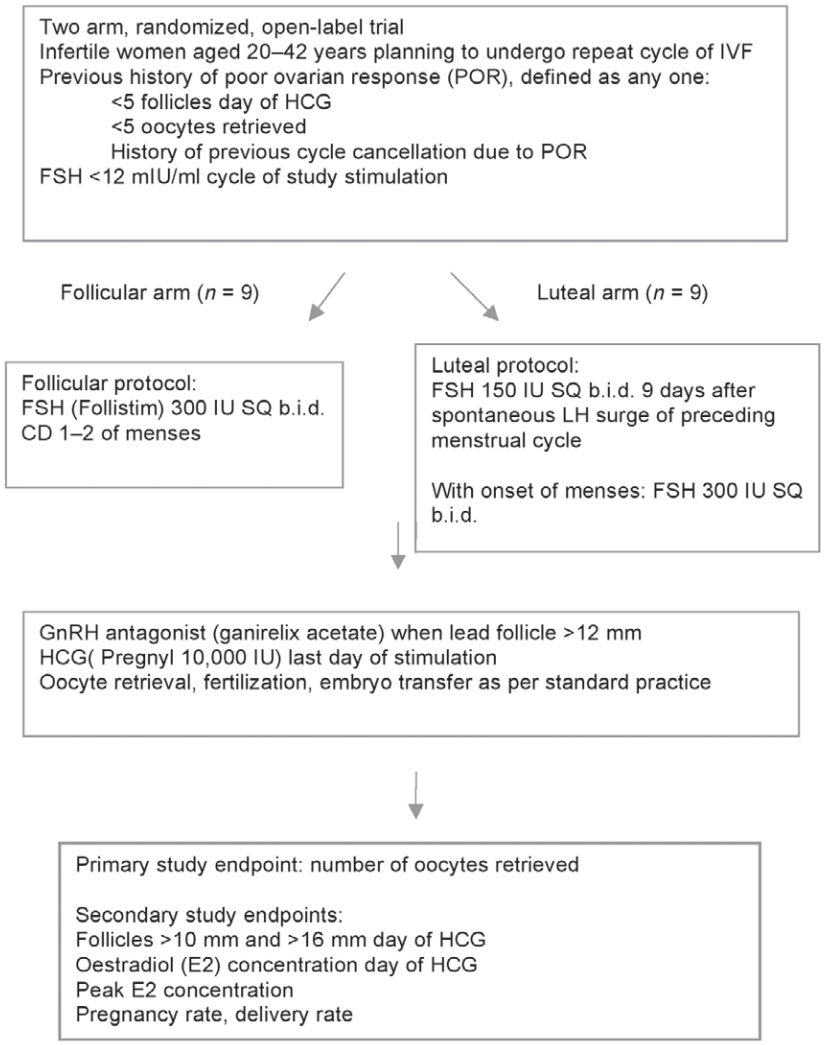

Figure 1. Flow chart of the study. b.i.d. = twice daily; GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin.

Mann–Whitney *U*- and chi-squared tests were used to compare the follicular and luteal protocols, and Wilcoxon signed rank test was used for paired analysis. $P < 0.05$ was considered statistically significant. As this was an intent-to-treat analysis, the all-treated population was examined and cancellation of treatment was evaluated as an outcome.

Results

The baseline characteristics of patients in the follicular and luteal protocols are presented in **Table 1**. As shown, age and FSH concentrations were similar in the two groups. In addition, the patient's response in the prior IVF cycle that met criteria for poor ovarian response was similar, including days of stimulation, total dose of gonadotrophin administered, peak oestradiol concentrations (which peaked on the day after HCG administration), oestradiol concentration on the day of HCG administration, follicles > 10 and 16 mm on the day of HCG, history of prior cycle cancellation, and number of oocytes retrieved.

Table 2 presents the response to stimulation in the follicular compared with the luteal protocol. The number of oocytes retrieved, the primary study endpoint, was similar in both protocols. No patients underwent retrieval during menses. Also similar between the two groups was: the total dose of gonadotrophin administered, the peak oestradiol concentration, the oestradiol concentration on the day of HCG administration, follicles >10 and >16 mm on the day of HCG, number of embryos transferred, cycle cancellation rate, and overall clinical pregnancy rate. However, the variability tended to be less in the luteal protocol, as shown by more narrow interquartile ranges for oestradiol concentrations, follicles on the day of HCG and retrieved oocytes in the luteal arm compared with the follicular arm. There was one cycle cancellation in the trial in the follicular arm, due to failure of pituitary suppression with premature LH surge during stimulation.

Overall, three patients randomized to the follicular phase protocol became pregnant, while one patient in the luteal phase protocol became pregnant. Of the three pregnancies in the

Table 1. Baseline characteristics of patients in follicular versus luteal phase FSH stimulation protocols.

Characteristic	*Follicular* (n=9)	*Luteal* (n=9)
Age (years)	36 (34–40)	35 (31–40)
Day 3 FSH	7.6 (3.7–11.3)	7.4 (5.7–10.7)
Previous cycle characteristics		
Days of stimulation	12 (7–12)	11 (9–12)
Total FSH dose (IU)	5700 (2250–9450)	5700 (2475–6900)
Peak oestradiol (pg/ml)	891 (58–1579)	875 (179–2509)
Oestradiol on day of HCG (pg/ml)	802 (653–1579)	893 (305–2462)
Follicles >10 mm on day of HCG	5 (2–7)	4 (1–11)
Follicles > 16 mm on day of HCG	2 (0–4)	3 (0–6)
History of cycle cancellation prior to USOR (%)	5/9 (55.6)	5/9 (55.6)
Number of oocytes retrieved if USOR[a]	3 (0–4)	2.5 (0–4)
Oocytes retrieved (including previous cycle cancellations)[b]	0 (0–4)	0 (0–4)

Values are median (range) unless otherwise stated. There were no statistically significant differences between the two protocols with respect to patient characteristics (Mann–Whitney *U*- or chi-squared test). USOR = ultrasound guided oocyte retrieval.
[a]Only 4/9 patients in each arm underwent ultrasound guided oocyte retrieval in a previous cycle.
[b]Compared with all patients in prior cycle (cancellation of prior cycle = 0 retrieved oocytes).

Table 2. Patient response to follicular versus luteal phase FSH stimulation.

Patient response	*Follicular* (n= 9)	*Luteal* (n = 9)
Days of stimulation	12 (11–15)	14 (11–17)
Total FSH dose (IU)	6900 (4800–8700)	6600 (2700–8400)
Peak oestradiol (pg/ml)	1180 (392–2792)	910 (539–1999)
Oestradiol on day of HCG (pg/ml)	965 (392–2194)	842 (494–1538)
Follicles >10 mm on day of HCG	8 (2–16)	8 (4–11)
Follicles >16 mm day of HCG	4 (1–12)	4 (2–7)
Number of oocytes retrieved	5.5 (1–14)	5.0 (3–8)
Endometrial stripe thickness on day of HCG	10.4 (8.2–13.0)	9.9 (8.0–14.7)
Number of embryos transferred	1 (0–4)	1 (0–4)
Cycle cancellation (%)	1/9 (11)	0/9 (0)
Clinical pregnancy rate (%)	3/9 (33.3)	1/9 (11.1)
Spontaneous pregnancy in cycle following stimulation	0/9 (0.0)	2/9 (22.2)
Live birth rate	2/9 (22.2)	0/9 (0.0)

Values are median (range) unless otherwise stated. There were no statistically significant differences between the two protocols with respect to patient response (Mann–Whitney *U*- or chi-squared test).

follicular phase protocol, one resulted in a pregnancy loss at 6 weeks' gestation and the other two resulted in normal term deliveries. The single pregnancy achieved with luteal phase initiation of FSH resulted in a second trimester pregnancy loss at 18 weeks following amniocentesis. In addition, there were two spontaneous pregnancies in natural cycles immediately following failed luteal protocol IVF; both of these pregnancies resulted in live births at term. There were no reported adverse safety endpoints in either protocol.

Paired analyses to evaluate each patient's response to IVF in the study cycle compared with the previous response to IVF were also performed. In the luteal arm, there was a statistically significant increase in days of stimulation (P = 0.01) and follicles >10 mm (P = 0.02) and >16 mm (P = 0.02) on day of HCG in the study cycle as compared with each patient's response in the IVF cycle that met criteria for inclusion in the study. In the follicular arm, the study cycle was only associated with more follicles >10 mm (P = 0.05) compared with each patient's baseline IVF cycle. Other stimulation parameters were similar to the previous cycle.

Discussion

This pilot study found that luteal phase initiation of FSH is a safe and potentially attractive alternative protocol in the IVF poor responder. However, no increase in oocyte yield was found (the primary study outcome) with luteal initiation of FSH compared with standard follicular phase FSH. The two protocols were also similar in terms of peak oestradiol concentrations, immature and mature follicle number on day of HCG, cycle cancellation rate, pregnancy rate or live birth rate. Of note, the luteal phase FSH regimen was associated

with narrower interquartile ranges, which suggests a potentially more consistent and predictable response in the poor responder. When paired analysis was performed, patients in the luteal arm had significantly more mature follicles (>16 mm) and immature follicles (>10 mm) present on the day of HCG compared with their prior cycle. This suggests that, as postulated, there were a greater number of follicles in the developing cohort, but this pilot study was unable to demonstrate an increase in oocyte yield or pregnancy.

Two of the three pregnancies following follicular phase initiation of FSH resulted in a live birth at term, while the one pregnancy achieved in the luteal arm resulted in an 18 week pregnancy loss. The occurrence of two spontaneously conceived term deliveries in the menstrual cycle immediately following luteal stimulation may suggest a potential carry-over effect. It is possible that the increased number of follicles in the developing cohort gave rise to pregnancies in the subsequent menstrual cycle. Of interest, the poor responder patients appeared to respond better than expected in terms of follicular development and oocyte yield (5.5 versus 5.0). It was attempted to identify true poor responders based on the definition. It is possible that the lack of a universal definition in the literature makes study of this group difficult and, as such, these findings reflect the inclusion of women who met the study criteria, but did not seem to represent a patient with true diminished ovarian response. The improved response seen in the patients may reflect a carryover effect of prior stimulation.

This is not the first proposal for luteo-follicular recruitment of follicles. Rombauts *et al.* (1998) performed a prospective, randomized, controlled trial examining the benefit of initiating luteal phase FSH therapy in an agonist cycle to assess whether expanding the window of recruitment would improve the number of oocytes retrieved. The present study differed in that the FSH dose chosen by the previous authors was only 150 IU once daily and it is widely accepted that poor responders require much higher doses of FSH to achieve stimulation (Padilla *et al.*, 1996), thus, the present study proposed using a higher dose of 300 IU twice a day after onset of menses to attempt to optimize response. In addition, GnRH antagonist was utilised for pituitary suppression instead of GnRH agonist. Despite these changes, this study was in agreement with the findings of Rombauts *et al.* in that a clear benefit of luteal phase FSH with antagonist compared with standard follicular phase FSH initiation was not demonstrated. A second, more recently published, study (Kucuk and Sozen, 2007) investigated luteal initiation of recombinant FSH with GnRH agonist versus short GnRH agonist protocol with initiation of FSH on cycle day 2 in patients with poor ovarian response undergoing intracytoplasmic sperm injection (ICSI) cycles. Although these authors did find a significant increase in the number of metaphase II oocytes, they did not find a statistically significant increase in clinical pregnancy rate with luteal initiation of FSH, further corroborating the present findings as well as those of Rombauts *et al.* (Kucuk and Sozen, 2007). It is possible that there are other aspects of the protocol that need to be investigated.

Patients with a history of poor ovarian response in a prior IVF cycle remain a challenging subset of patients to treat. Many protocols have been proposed and investigated in poor IVF responders. A recent Cochrane review concluded that there was a lack of evidence to support any particular IVF regimen in patients with a history of poor ovarian response (Shanbhag *et al.*, 2007). However, the literature comparing protocols in poor responders is limited and, therefore, difficult to interpret (Griesinger *et al.*, 2006). For instance, studies are hindered by lack of a universal definition of poor ovarian response in IVF, lack of randomization, inclusion of differing doses of gonadotrophins, analysis of surrogate outcomes and small sample sizes (Faber *et al.*, 1998; Wang *et al.*, 2002; Eskander *et al.*, 2004; Klinkert *et al.*, 2005; Barrenetxea *et al.*, 2008). As such, a protocol that conclusively demonstrates increased pregnancy and, more importantly, live birth rates in the IVF patient with a history of poor ovarian response remains elusive.

In summary, luteal phase initiation of FSH with GnRH antagonist appears to be a safe alternative in patients with poor ovarian response. Although no clear benefit of this protocol was discerned in comparison with a standard GnRH antagonist protocol initiating FSH in the follicular phase, it may be considered as an alternative when other protocols have failed. The narrower interquartile ranges for oocyte yield and oestradiol concentrations in the luteal arm suggest a potentially more consistent, predictable response. In addition, the two spontaneous pregnancies immediately following stimulation with luteal FSH and the increased number of mature and immature follicles in paired analysis suggest that there may be a potential carryover effect. Given the findings of this pilot study, it is concluded that luteal phase initiation of FSH holds promise and should be further investigated. Well-designed, prospective, randomized, larger-scale studies that utilize a universal definition of poor response and assess the clinically relevant endpoint of live birth need to be performed to better elucidate the optimal stimulation regimen for this challenging group of patients.

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