Reproductive BioMedicine Online (2014) 28, 359–368

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Impact of clomiphene citrate during ovarian stimulation on the luteal phase after GnRH agonist trigger

L Derksen [a], H Tournaye [a], D Stoop [a], I Van Vaerenbergh [b], C Bourgain [b], NP Polyzos [a], P Haentjens [c], C Blockeel [a,*]

[a] *Centre for Reproductive Medicine, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium*; [b] *Reproductive Immunology and Implantation, Vrije Universiteit Brussel, Brussels, Belgium*; [c] *Centre for Outcomes Research and Laboratory for Experimental Surgery, UZ Brussel, Brussels, Belgium*
[*] Corresponding author. *E-mail address:* christophe.blockeel@uzbrussel.be (C Blockeel).

Lotte Derksen, born in 1990 in the Netherlands, is a medical student at the Radboud University, Nijmegen. She has a BSc in medical biology and wrote her master thesis at the Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel. Currently, she is engaged in obtaining a MSc in medicine. Her major interest is reproductive medicine and general gynaecology.

Abstract The use of a gonadotrophin-releasing hormone (GnRH) agonist to trigger final oocyte maturation in a GnRH antagonist protocol has been associated with poorer clinical outcomes due to an increased luteal-phase defect. It has been shown that LH activity is crucial in a normal luteal phase. Studies assessing the LH concentrations after clomiphene citrate co-treatment have observed increased luteal-phase LH concentrations. The purpose of this prospective cohort study was to analyse the effect of clomiphene citrate on the endocrine profile in the luteal phase when using GnRH agonist trigger. This was evaluated in eight oocyte donors undergoing ovarian stimulation using clomiphene citrate in combination with recombinant FSH compared with a control group of five donors treated with recombinant FSH only. The endocrine profile was comparable in both groups, except for serum LH concentrations on the day after trigger (121.3 ± 53.0 IU/l versus 52.9 ± 21.5 IU/l, respectively, $P = 0.022$). No significant differences in LH concentrations were found on the day of trigger or 5 days after oocyte retrieval. In conclusion, a luteal-phase defect was observed despite treatment with clomiphene citrate during ovarian stimulation.

KEYWORDS: clomiphene citrate, GnRH agonist trigger, GnRH antagonist, ovarian stimulation, recombinant FSH, IVF/ICSI

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.10.019

Introduction

For many years, human chorionic gonadotrophin (HCG) has played a crucial role at the end of the ovarian stimulation in an IVF or intracytoplasmic sperm injection (ICSI) cycle. It has been the gold standard for triggering final oocyte maturation as a substitute for the endogenous LH surge (Macklon et al., 2006). Nevertheless, induction of final oocyte maturation with HCG is believed to play a key role in the development of ovarian hyperstimulation syndrome (OHSS) (Elchalal and Schenker, 1997; Goldsman et al., 1995; Whelan and Vlahos, 2000). Human chorionic gonadotrophin (HCG) has a significantly longer half-life compared with LH, which results in a prolonged luteotrophic effect, development of multiple corpora lutea and raised steroid concentrations in the luteal phase (Damewood et al., 1989; Humaidan et al., 2010b; Itskovitz et al., 1991). Additionally, it has been suggested that HCG has an adverse impact on endometrial receptivity (Fatemi et al., 2010).

In order to prevent OHSS, gonadotrophin-releasing hormone (GnRH) antagonists have been increasingly used in assisted reproduction treatment clinics worldwide, since they are associated with a significantly lower probability of hospital admission due to OHSS (Kolibianakis et al., 2006). Besides the use of GnRH antagonists, alternative agents for triggering final oocyte maturation in these protocols have been postulated. For more than two decades, GnRH agonists have proven to effectively induce ovulation, with an LH and FSH surge similar to that of a natural cycle (Gonen et al., 1990; Itskovitz et al., 1991). With the introduction of GnRH antagonists to prevent a premature LH surge, triggering final oocyte maturation with a GnRH agonist as an alternative for HCG has gained renewed interest. Due to the shorter half-life, GnRH agonist triggering reduces or even eliminates the risk of OHSS (Kol, 2004; Kol and Itskovitz-Eldor, 2000; Orvieto, 2005). Besides, different studies have observed an increased number of oocytes retrieved compared with HCG trigger (Humaidan et al., 2005; Imoedemhe et al., 1991; Oktay et al., 2010). Regardless of these clear advantages of using GnRH agonist for final oocyte maturation, several randomized trials have reported poor clinical outcomes (Griesinger et al., 2006; Humaidan et al., 2005; Kolibianakis et al., 2005). These poor results were attributed to a luteal-phase defect, despite standard luteal-phase support (Humaidan et al., 2005; Kolibianakis et al., 2005). Therefore, a freeze-all strategy should be applied in these patients (Devroey et al., 2011; Garcia-Velasco, 2012). Nevertheless, different treatment strategies for luteal-phase rescue have been postulated, resulting in a comparable clinical outcome to that seen after HCG triggering (Humaidan et al., 2011). The luteal phase can be rescued with a bolus of 1500 IU HCG after oocyte retrieval (Humaidan et al., 2006, 2010a; Humaidan, 2012) and the use of intensive luteal-phase support with intramuscular progesterone and oestradiol patches also achieved good clinical outcomes (Engmann and Benadiva, 2012; Engmann et al., 2008).

LH activity plays a crucial role in the luteal phase, since it is responsible for the maintenance of the corpus luteum and its steroidogenic activity (Casper and Yen, 1979). Furthermore, LH is also involved in the activation of extragonadal LH receptors in the endometrium and the up-regulation of growth factors involved in implantation (Licht et al., 2001; Rao, 2001; Sugino et al., 2000; Tesarik et al., 2003; Wang et al., 2002). Subsequently, low LH concentrations in the luteal phase impair implantation and corpus luteum function (Duffy et al., 1999). Triggering ovulation with a GnRH agonist results in reduction of LH and FSH secretion after the initial flare-up effect due to the desensitizing effect (Gonen et al., 1990; Itskovitz et al., 1991). The decreased endogenous LH activity seen after GnRH agonist triggering, could explain the poor clinical results reported (Humaidan et al., 2012).

Two studies assessing the LH concentrations in the luteal phase after administration of clomiphene citrate in the follicular phase, reported higher luteal-phase LH concentrations than control groups treated with gonadotrophins (Smitz et al., 1990; Tavaniotou et al., 2002). Clomiphene citrate is an oral anti-oestrogen which is highly effective in inducing ovulation and is relatively safe and inexpensive (Casper and Mitwally, 2006). It raises gonadotrophin secretion due to blockage of the oestradiol receptor in the hypothalamus, which generally induces the development of two or more follicles (Macklon et al., 2006).

Due to the higher LH concentrations reported after treatment with clomiphene citrate, this work postulated that the use of clomiphene citrate in the early follicular phase could provide sufficient LH concentrations in the luteal phase when GnRH agonist is used for final oocyte maturation. The purpose of the current prospective cohort study is to analyse the effect of administration of clomiphene citrate during ovarian stimulation on the luteal phase when using GnRH agonist triggering. This work studied endocrine profiles and performed histological evaluations of the endometrium.

Materials and methods

Study design

This study was conducted in 13 oocyte donors, all included between 2009 and 2011. A prospective cohort design was used to analyse the effect of clomiphene citrate treatment in addition to recombinant FSH on the luteal phase in terms of endocrine and histological profile. As parallel controls, a cohort of oocyte donors undergoing standard ovarian stimulation with recombinant FSH was used.

All participants were recruited from the IVF outpatient clinic of the Centre for Reproductive Medicine of the Universitair Ziekenhuis Brussel. This study received institutional review board approval by the local Institute Ethics Committee (BUN 14320108837, approved 24 June 2010). Written informed consent was provided by all subjects.

Study population

Oocyte donor patients younger than 36 years, with a body mass index 18–29 kg/m^2 (both inclusive), a regular menstrual cycle of 21–35 days, FSH concentration $<$12 IU/l on cycle day 2 and a normal ultrasound scan were eligible to be enrolled in the study. Patients who had uterine or ovarian abnormalities, polycystic ovary syndrome, endocrine or metabolic abnormalities or endometriosis (grade 3 or more) were excluded. Another exclusion criterion was a low

response to ovarian stimulation (less than four follicles) in a preceding IVF/ICSI donor cycle.

Stimulation protocol

In both treatment groups, a standard GnRH antagonist protocol was applied, involving daily recombinant FSH injections (Puregon; MSD, Oss, The Netherlands) initiated at a dose of 200 IU on cycle day 2. The clomiphene citrate group received additional stimulation with daily administration of 100 mg clomiphene citrate (Clomid; Sanofi-Aventis, Brussels, Belgium) from cycle day 2 until day 6. In both treatment groups, administration of the GnRH antagonist ganirelix (Orgalutran; MSD) was initiated on cycle day 7 at a daily dose of 0.25 mg to prevent a premature LH surge. Suppression with GnRH antagonist was continued until the day of final oocyte maturation. An outline of both treatment groups is presented in Figure 1.

A single injection of 0.2 mg triptorelin (Decapeptyl; Ferring Pharmaceuticals, Copenhagen, Denmark) was administered to induce final oocyte maturation as soon as three follicles of $\geq$17 mm were present on the ultrasonography. Oocyte retrieval was performed 36 h after GnRH agonist administration. The oocyte donors did not receive any luteal-phase support.

Hormone assays

Hormonal analyses were performed on five different days: cycle day 1–2, cycle day 5–6, on the day of GnRH agonist trigger, 1 day after GnRH agonist trigger and 5 days after oocyte retrieval. Triggering of final oocyte maturation took place between 0900 and 2300 hours, since the oocyte retrieval of the oocyte donors takes place between 0900 and 1100 hours (namely 36 h after trigger). Therefore, the blood collection took place between 8 and 12 h after trigger (i.e. 1 day after GnRH agonist trigger). Automated immune analysis was performed to measure serum concentrations of FSH, LH, oestradiol and progesterone. This was done by the hormone laboratory at Universitair Ziekenhuis Brussel (Brussels, Belgium) by validated laboratory immunoassay methods (electrochemiluminescence; Cobas 6000; Roche, Indianapolis, IN, USA).

Endometrial biopsy and histological assessment

Aspirational biopsy of the endometrium was performed using the Pipelle de Cornier (CCD International, Paris, France) 5 days after oocyte retrieval. The biopsies were divided into two pieces. One part of the endometrial tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin and cut into 4-μm-thick sections for histological analysis with haematoxylin and eosin staining. The other part was snap frozen in liquid nitrogen for further RNA isolation. Endometrial dating was performed on all samples by a specialized pathologist, blinded for the used stimulation protocol and according to the criteria of Noyes et al. (1975).

Endpoints

The primary endpoint of this study was the hormonal profile during luteal phase. The secondary endpoint was the number of oocytes retrieved.

Statistical analysis

Data on stimulation characteristics, embryological characteristics and hormone laboratory values are summarized

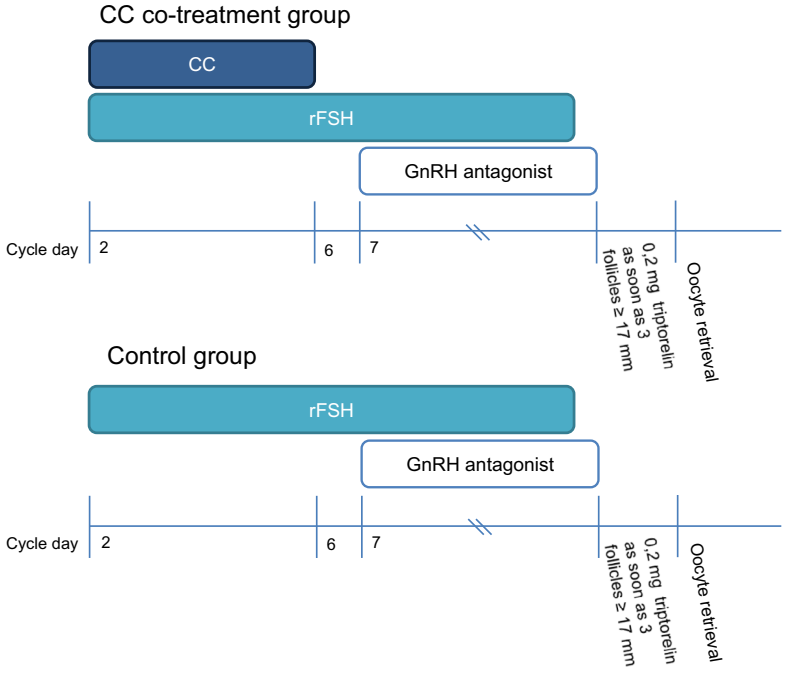

Figure 1 Graphical illustration of the treatment regimen applied in this prospective cohort design. CC = clomiphene citrate; GnRH = gonadotrophin-releasing hormone; rFSH = recombinant FSH.

descriptively for both cohorts (i.e. oocyte donors with or without clomiphene citrate co-stimulation). Continuous data are presented as mean ± standard deviation (SD). Stimulation characteristics and embryologic characteristics were compared by means of unpaired Student's t-tests. The comparative analysis of endocrine profile in the stimulated cohort versus the control cohort was carried out using both repeated measures ANOVA and unpaired Student's t-tests.

A one-way repeated measures ANOVA mixed between-within subject analysis was conducted to assess the overall impact of clomiphene citrate treatment on participants' endocrine profile, across three time periods in the luteal phase (day of trigger, 1 day after trigger, 5 days after trigger). Four hormones — FSH, LH, oestradiol and progesterone — were modelled separately. To detect subtle differences between the two cohorts, unpaired Student's t-tests were carried out at each time point for each hormone separately. Level of significance was $P < 0.05$.

All computational procedures were performed using Excel 2003 (Microsoft Office, 2003) and IBM SPSS Statistics version 20 (IBM Corporation, 2012).

Results

Clinical results

There was no significant difference between the two groups with regard to demographic characteristics (i.e. mean age, bodyweight, height and body mass index (Table 1). Total dose of recombinant FSH consumed and duration of stimulation did not significantly differ between both groups. The number of cumulus—oocyte complexes obtained at retrieval

Table 1 Baseline and stimulation characteristics and clinical outcome measures.

	Clomiphene citrate (n = 8)	*Control* (n = 5)
Demographics		
Age (years)	27.9 ± 5.2	25.4 ± 2.2
Bodyweight (kg)	70.3 ± 11.8	77.5 ± 20.8
Height (m)	1.7 ± 0.1	1.7 ± 0.0
Body mass index (kg/m^2)	22.9 ± 3.2	27.9 ± 6.6
Stimulation		
Total recombinant FSH (IU)	2025.0 ± 423.4	1920 ± 228.0
Duration of stimulation (days)	10.3 ± 2.1	9.6 ± 1.1
Embryological outcome		
Cumulus—oocyte complexes	17.1 ± 6.2	15.2 ± 10.0
Metaphase-II oocytes	12.8 ± 5.3	12.4 ± 7.5

Data are mean ± SD. No statistically significant between-group differences were found (Student's t-test).

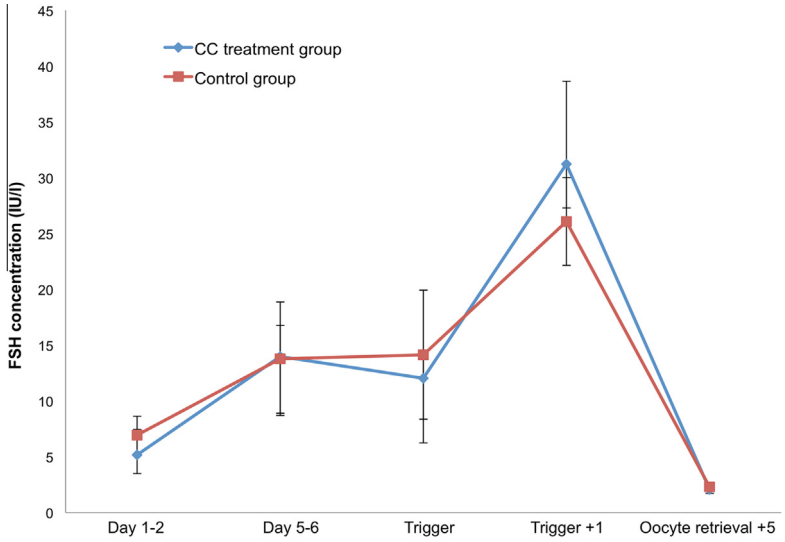

Figure 2 FSH concentrations in the clomiphene citrate (CC) and control groups on cycle days 1—2 and 5—6, day of GnRH agonist trigger, the day after GnRH agonist trigger and 5 days after oocyte retrieval. Values are mean ± SD.

Table 2 Stimulation characteristic and clinical outcome measures.

	CC co-treatment group (n = 8)	*Control group (n = 5)*	P-*value*[b]
Stimulation characteristics[a]			
Total dose of rFSH (IU)	2025.0 ± 423.4	1920 ± 228.0	0.62
Total duration of stimulation (days)	10.3 ± 2.1	9.6 ± 1.1	0.53
Embryological characteristics[a]			
Number of COCs	17.1 ± 6.2	15.2 ± 10.0	0.67
Number of MII oocytes	12.8 ± 5.3	12.4 ± 7.5	0.92

COC = Cumulus—oocyte complex, MII = metaphase II.
[a]Data are presented as mean ± SD.
[b]*P*-value for between-group difference from Student's *t*-test.

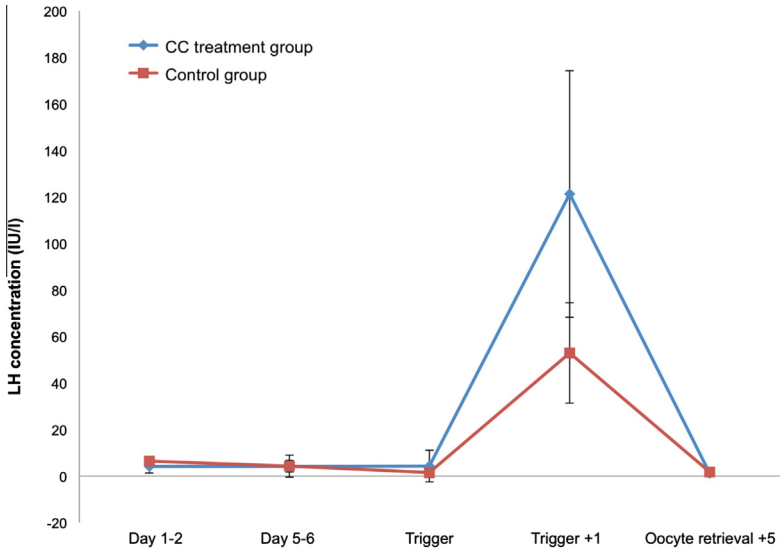

Figure 3 LH concentrations in the clomiphene citrate (CC) and control groups on cycle days 1—2 and 5—6, day of GnRH agonist trigger, the day after GnRH agonist trigger and 5 days after oocyte retrieval. Values are mean ± SD.

was comparable in both treatment groups, as well as the number of MII oocytes (Table 1).

In the clomiphene citrate group, six out of the eight included donors were referred to the study centre's egg bank, so no further details regarding performance nor outcome can be provided yet. Of the two remaining oocyte donors in the clomiphene citrate group, one oocyte recipient became pregnant and delivered. In the FSH group, all five donors were 'fresh donations', and oneoocyte donor gave her oocytes to two recipients. Four out of six oocyte recipients became pregnant, but one pregnancy was biochemical and another pregnancy ended in a miscarriage.

Endocrine profile

FSH

FSH concentrations did not differ between the treatment protocols neither on cycle day 1—2 nor on day 5—6 (Figure 2 and Table 2). In the luteal phase, the main effect comparing the two types of intervention was not significant (ANOVA). On the day of ovulation trigger, 1 day after trigger and 5 days after oocyte retrieval, the FSH concentrations were comparable between both groups.

LH

In the follicular phase on days 1—2 and 5—6, no significant difference was found between both groups (Figure 3 and Table 2). In the luteal phase, the main effect comparing the two types of intervention was not statistically different (ANOVA). Although no significant differences were found neither on the day of ovulation trigger nor 5 days after oocyte retrieval, LH concentrations on the day after trigger were significantly higher in the clomiphene citrate group ($P = 0.022$).

Oestradiol

Oestradiol concentrations were comparable between the two protocols on cycle day 5—6; however, on cycle day 1—2 the oestradiol concentration was significantly lower in the clomiphene citrate group ($P = 0.014$; Figure 4 and Table 2). In the luteal phase, the main effect comparing the two types of intervention was not significant (ANOVA).

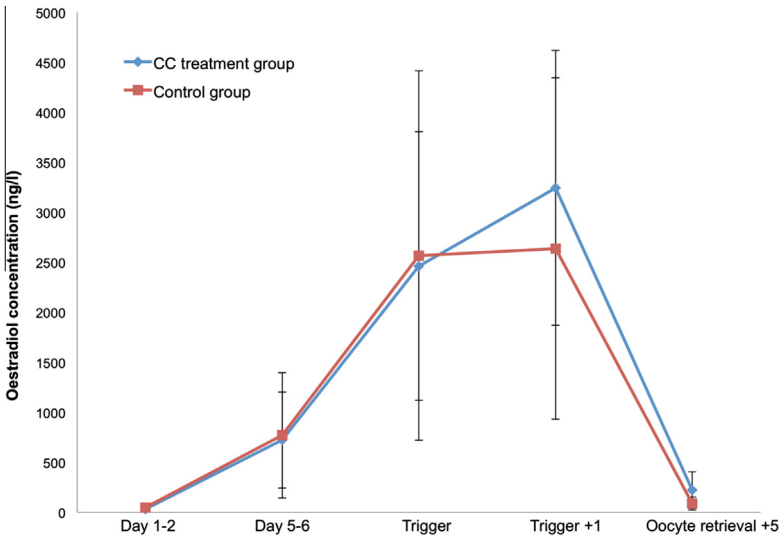

Figure 4 Oestradiol concentrations in the clomiphene citrate (CC) in and control groups on cycle days 1—2 and 5—6, day of GnRH agonist trigger, the day after GnRH agonist trigger and 5 days after oocyte retrieval. Values are mean ± SD.

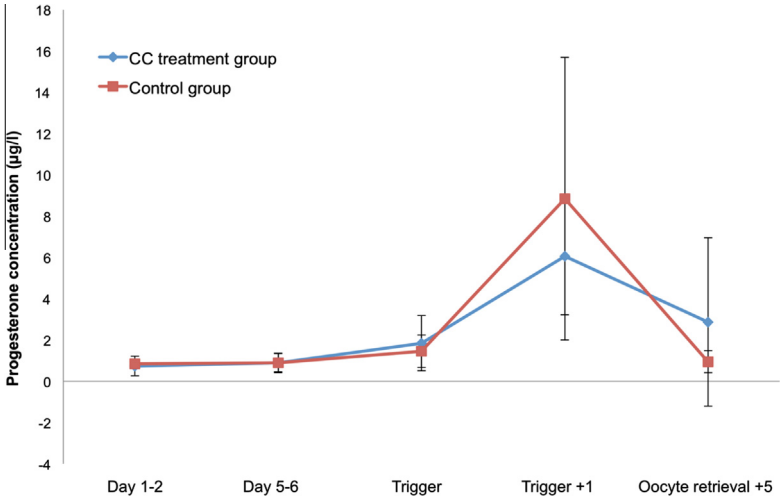

Figure 5 Progesterone concentrations in the clomiphene citrate (CC) in and control groups on cycle days 1—2 and 5—6, day of GnRH agonist trigger, the day after GnRH agonist trigger and 5 days after oocyte retrieval. Values are mean ± SD.

On the day of ovulation trigger, 1 day after trigger and 5 days after oocyte retrieval, oestradiol concentrations were comparable between both groups.

Progesterone

Progesterone concentrations did not differ between the treatment protocols neither on cycle day 1—2 nor on day 5—6 (Figure 5 and Table 2). In the luteal phase, the main effect comparing the two types of intervention was not significant (ANOVA). Progesterone concentrations were similar between both groups when measured on the day of ovulation trigger, 1 day after trigger and 5 days after oocyte retrieval.

Endometrial thickness and histology

The thickness of the endometrium on the day of triggering was (mean ± SD) 9.1 ± 4.1 mm in the clomiphene citrate group and 9.4 ± 1.5 mm in the control group. Histological results of the endometrial biopsy on the day of oocyte retrieval are shown in Table 3. The majority of the oocyte donors showed endometrial advancement on histology (Table 4).

Discussion

As far as is known, this prospective cohort study is the first study analysing the effect of clomiphene citrate treatment during ovarian stimulation on the luteal phase when using GnRH agonist for final oocyte maturation. This study reports only a subtle impact of clomiphene citrate on the endocrine profile in luteal phase.

The luteal phase is known to be abnormal in all the IVF cycles involving ovarian stimulation (Edwards et al., 1980). It is assumed that this luteal-phase defect is associated with the supra-physiological concentrations of oestradiol and

Table 3 Endocrine profiles in the follicular and luteal phases.

	Clomiphene citrate (n = 8)	*Control* (n = 5)
Cycle day 1—2		
FSH (IU/l)	5.2 ± 2.3	6.9 ± 1.7
LH (IU/l)	4.1 ± 2.8	6.3 ± 1.6
Oestradiol (ng/l)	30.8 ± 14.8[a]	48.0 ± 4.9[a]
Progesterone (μg/l)	0.7 ± 0.5	0.8 ± 0.2
Cycle day 5—6		
FSH (IU/l)	14.0 ± 2.8	13.8 ± 5.1
LH (IU/l)	4.2 ± 2.5	4.3 ± 4.7
Oestradiol (ng/l)	722.7 ± 479.6	771.0 ± 628.8
Progesterone (μg/l)	0.9 ± 0.4	0.9 ± 0.5
Day of trigger		
FSH (IU/l)	12.0 ± 2.5	14.1 ± 5.8
LH (IU/l)	4.3 ± 6.8	1.5 ± 2.3
Oestradiol (ng/l)	2463.5 ± 1343.5	2568.6 ± 1848.8
Progesterone (μg/l)	1.8 ± 1.3	1.5 ± 0.8
One day after trigger		
FSH (IU/l)	31.2 ± 7.4	26.1 ± 3.9
LH (IU/l)	121.3 ± 53.0[b]	52.9 ± 21.5[b]
Oestradiol (ng/l)	3247.0 ± 1375.6	2639.6 ± 1709.2
Progesterone (μg/l)	6.1 ± 2.8	8.8 ± 6.8
Five days after retrieval		
FSH (IU/l)	2.1 ± 0.6	2.3 ± 0.4
LH (IU/l)	1.2 ± 1.1	1.7 ± 0.7
Oestradiol (ng/l)	221.0 ± 184.5	87.0 ± 64.7
Progesterone (μg/l)	2.9 ± 4.1	0.9 ± 0.5

Values are mean ± SD.
[a,b]*P*-values for between-group difference from Student's t-tests: [a]*P* = 0.014; [b]*P* = 0.022.

Table 4 Histological dating of the endometrial biopsy.

	Clomiphene citrate (n = 8)	*Control* (n = 5)
Early secretory phase	0	0
Mid secretory phase	5	1
Late secretory phase	0	1
Out of phase	0	1
Insufficient sample for diagnosis	3	2

Early secretory phase = luteal-phase days 0—4; mid secretory phase = luteal-phase days 5—9; late secretory phase = luteal-phase days 10—14.

progesterone due to multifollicular development (Fatemi et al., 2007). The high steroid concentrations have a negative feedback on the hypothalamic—pituitary axis, resulting in suppression of LH secretion. LH activity plays a crucial role in the luteal phase, since it stimulates implantation and is entirely responsible for the steroid activity of the corpus luteum (Casper and Yen, 1979).

In this report, it was postulated that co-treatment with clomiphene citrate may result in sufficient LH concentrations in the luteal phase to adequately support the corpus luteum. Clomiphene citrate binds to the oestrogen receptor much longer than oestrogen, which might explain the higher luteal-phase LH concentrations reported in clomiphene citrate stimulated cycles (Smitz et al., 1990; Tavaniotou et al., 2002). In a study using clomiphene citrate with GnRH agonist for ovulation induction in anovulatory patients, physiological FSH concentrations and a normal LH surge were observed together with a normal luteal phase, despite the absence of luteal-phase support. Moreover, no significant difference in either pregnancy rate or abortion rate was

noted compared with HCG-triggered cycles (Shalev et al., 1995). In contrast, a similar study in which patients were treated with gonadotrophin and a GnRH agonist trigger did not report a normal luteal phase (Emperaire et al., 2004), and the authors attributed this inconsistency to the clomiphene citrate stimulation used by Shalev et al. However, in the current prospective cohort design with a parallel control cohort, a similar response to clomiphene citrate administration in ovarian stimulation cycles could not be demonstrated.

The results of the current prospective cohort study show higher LH concentrations in the clomiphene citrate group only on the day after GnRH agonist trigger, but not 5 days after oocyte retrieval. The main effect comparing the two types of intervention was not significantly different for LH (ANOVA). The total drop in LH as well as FSH concentrations in the luteal phase may result from the immediate desensitizing effect after the flare-up of GnRH agonist (Fauser et al., 2002). Apparently, co-treatment with clomiphene citrate in early follicular phase did not counteract this effect. Nevertheless, it needs to be mentioned that there is no significant difference in the LH concentrations on day 5 after agonist or HCG trigger (Fauser et al., 2002). Therefore, luteal LH concentrations alone cannot explain the abrupt luteolysis.

In contrast to HCG trigger, using GnRH agonist for final oocyte maturation induces a rise of FSH concentrations in addition to LH (Humaidan et al., 2011). The current study observed a rise in LH and FSH in both groups. It has been postulated that the FSH surge associated with GnRH agonist trigger results in a higher number of oocytes retrieved compared with HCG trigger (Imoedemhe et al., 1991; Humaidan et al., 2009, 2005). The function of the mid-cyclic FSH surge is not fully understood, but may play a role in final nuclear maturation (Yding Andersen et al., 1999; Zelinski-Wooten et al., 1995). Co-treatment with clomiphene citrate in the follicular phase did not affect FSH secretion as no significant difference was found between both study groups. Yet, higher FSH concentrations in the follicular phase were expected in the clomiphene citrate group, treated with clomiphene citrate in addition to the standard ovarian stimulation.

A luteal-phase defect is characterized by elevated progesterone concentrations in the early luteal phase in combination with a shorter luteal phase (Jones, 1996). Since the current work aimed to prevent a luteal-phase defect by adding clomiphene citrate to the ovarian stimulation in the follicular phase, lower serum progesterone concentrations in the early luteal phase and higher concentrations in the late luteal phase were expected in the clomiphene citrate group. However, progesterone concentrations were similar throughout both the follicular and the luteal phase.

The oestradiol concentrations were similar throughout the stimulation phase, except on day 1–2 of the cycle when they were significantly lower in the clomiphene citrate group (**Table 2**), although this difference has no clinical importance and is probably due to the low sample size.

The majority of the studies performed in cycles stimulated with clomiphene/human menopausal gonadotrophin observed abnormal endometrial development (Cohen et al., 1984; Kolibianakis and Devroey, 2002; Martel et al., 1987; Sharma et al., 1990). The results of the current prospective cohort study are in accordance with these studies, since endometrial advancement was fouind in the majority of the patients treated with clomiphene citrate/recombinant FSH. Endometrial advancement on the day of oocyte retrieval has been reported to decrease the chance of pregnancy (Kolibianakis et al., 2002). In addition, menstrual bleeding occurred 5–8 days after oocyte retrieval in all the patients treated with clomiphene citrate, which implies a decreased length of the luteal phase.

Maruncic and Casper (1987) assessed the effect of clomiphene citrate administration during the mid-luteal phase on the LH pulses. They observed an increased LH pulse frequency after administration of clomiphene citrate compared with the control group, resulting in significantly higher oestradiol and progesterone concentrations, and a significantly prolonged luteal phase. These findings suggest that clomiphene citrate administered after GnRH agonist trigger, instead of in the follicular phase like in this study, could possibly rescue the luteal phase.

Another possibility is the prolonged administration of clomiphene citrate, which could save the luteal phase. Teramoto and Kato (2007) conducted a large retrospective trial using the minimal ovarian stimulation protocol with the administration of clomiphene citrate initiated on day 3 and continued until the day before maturation triggering with the GnRH agonist. Administration of human menopausal gonadotrophin or FSH was initiated on day 8 at 150 IU per session and given to the patient every other day. Clomiphene citrate was used for inhibiting the premature LH surge while maintaining pituitary function and not to work as an anti-oestrogen in order to increase endogenous FSH. The authors observed a normal luteal phase with acceptable pregnancy rates.

A major limitation of this study is its nonrandomized design and the limited sample size. Therefore, results must be interpreted with caution. Future randomized trials need to be adequately powered to confirm the present findings. Besides, the endocrine profile was assessed during one time point in the luteal phase, namely on day 5 after oocyte retrieval. Tavaniotou et al. (2002) provide data from the early, mid and late luteal phase to obtain supplementary data.

The main finding in this parallel cohort study is a luteal defect in the clomiphene citrate group despite the treatment with clomiphene citrate in the follicular phase. This is demonstrated by the lack of sufficient LH concentrations in the luteal phase, the similar progesterone concentrations in both treatment arms and the endometrial advancement in the majority of the patients. Therefore, additional treatment with clomiphene citrate seems not to be a valid alternative for standard treatment, and adequate luteal-phase support after GnRH agonist triggering remains obligatory.

In conclusion, this study shows that the endocrine profile is only slightly affected and that endometrial advancement is present when adding clomiphene citrate to ovarian stimulation in a GnRH antagonist protocol using a GnRH agonist trigger. Clomiphene citrate co-treatment does not prevent the luteal-phase defect associated to GnRH agonist triggering.

References

Casper, R.F., Mitwally, M.F., 2006. Review: aromatase inhibitors for ovulation induction. J. Clin. Endocrinol. Metab. 91, 760—771.

Casper, R.F., Yen, S.S., 1979. Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. Science 205, 408—410.

Cohen, J.J., Debache, C., Pigeau, F., Mandelbaum, J., Plachot, M., de Brux, J., 1984. Sequential use of clomiphene citrate, human menopausal gonadotropin, and human chorionic gonadotropin in human in vitro fertilization. II. Study of luteal phase adequacy following aspiration of the preovulatory follicles. Fertil. Steril. 42, 360—365.

Damewood, M.D., Shen, W., Zacur, H.A., Schlaff, W.D., Rock, J.A., Wallach, E.E., 1989. Disappearance of exogenously administered human chorionic gonadotropin. Fertil. Steril. 52, 398—400.

Devroey, P., Polyzos, N.P., Blockeel, C., 2011. An OHSS-free clinic by segmentation of IVF treatment. Hum. Reprod. 26, 2593—2597.

Duffy, D.M., Stewart, D.R., Stouffer, R.L., 1999. Titrating luteinizing hormone replacement to sustain the structure and function of the corpus luteum after gonadotropin-releasing hormone antagonist treatment in rhesus monkeys. J. Clin. Endocrinol. Metab. 84, 342—349.

Edwards, R.G., Steptoe, P.C., Purdy, J.M., 1980. Establishing full-term human pregnancies using cleaving embryos grown in vitro. Br. J. Obstet. Gynaecol. 87, 737—756.

Elchalal, U., Schenker, J.G., 1997. The pathophysiology of ovarian hyperstimulation syndrome — views and ideas. Hum. Reprod. 12, 1129—1137.

Emperaire, J.C., Parneix, I., Ruffie, A., 2004. Luteal phase defects following agonist-triggered ovulation: a patient-dependent response. Reprod. Biomed. Online 9, 22—27.

Engmann, L., Benadiva, C., 2012. Agonist trigger: what is the best approach? Agonist trigger with aggressive luteal support. Fertil. Steril. 97, 531—533.

Engmann, L., DiLuigi, A., Schmidt, D., Nulsen, J., Maier, D., Benadiva, C., 2008. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. Fertil. Steril. 89, 84—91.

Fatemi, H.M., Popovic-Todorovic, B., Papanikolaou, E., Donoso, P., Devroey, P., 2007. An update of luteal phase support in stimulated IVF cycles. Hum. Reprod. Update 13, 581—590.

Fatemi, H.M., Kyrou, D., Bourgain, C., Van den Abbeel, E., Griesinger, G., Devroey, P., 2010. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. Fertil. Steril. 94, 2054—2058.

Fauser, B.C., de Jong, D., Olivennes, F., Wramsby, H., Tay, C., Itskovitz-Eldor, J., van Hooren, H.G., 2002. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. J. Clin. Endocrinol. Metab. 87, 709—715.

Garcia-Velasco, J.A., 2012. Agonist trigger: what is the best approach? Agonist trigger with vitrification of oocytes or embryos. Fertil. Steril. 97, 527—528.

Goldsman, M.P., Pedram, A., Dominguez, C.E., Ciuffardi, I., Levin, E., Asch, R.H., 1995. Increased capillary permeability induced by human follicular fluid: a hypothesis for an ovarian origin of the hyperstimulation syndrome. Fertil. Steril. 63, 268—272.

Gonen, Y., Balakier, H., Powell, W., Casper, R.F., 1990. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization. J. Clin. Endocrinol. Metab. 71, 918—922.

Griesinger, G., Diedrich, K., Devroey, P., Kolibianakis, E.M., 2006. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum. Reprod. Update 12, 159—168.

Humaidan, P., 2012. Agonist trigger: what is the best approach? Agonist trigger and low dose HCG. Fertil. Steril. 97, 529—530.

Humaidan, P., Bredkjaer, H.E., Bungum, L., Bungum, M., Grondahl, M.L., Westergaard, L., Andersen, C.Y., 2005. GnRH agonist (buserelin) or HCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum. Reprod. 20, 1213—1220.

Humaidan, P., Bungum, L., Bungum, M., Yding Andersen, C., 2006. Rescue of corpus luteum function with peri-ovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: a pilot study. Reprod. Biomed. Online 13, 173—178.

Humaidan, P., Papanikolaou, E.G., Tarlatzis, B.C., 2009. GnRHa to trigger final oocyte maturation: a time to reconsider. Hum. Reprod. 24, 2389—2394.

Humaidan, P., Ejdrup Bredkjaer, H., Westergaard, L.G., Yding Andersen, C., 2010a. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. Fertil. Steril. 93, 847—854.

Humaidan, P., Quartarolo, J., Papanikolaou, E.G., 2010b. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. Fertil. Steril. 94, 389—400.

Humaidan, P., Kol, S., Papanikolaou, E.G., 2011. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum. Reprod. Update 17, 510—524.

Humaidan, P., Papanikolaou, E.G., Kyrou, D., Alsbjerg, B., Polyzos, N.P., Devroey, P., Fatemi, H.M., 2012. The luteal phase after GnRH-agonist triggering of ovulation: present and future perspectives. Reprod. Biomed. Online 24, 134—141.

Imoedemhe, D.A., Sigue, A.B., Pacpaco, E.L., Olazo, A.B., 1991. Stimulation of endogenous surge of luteinizing hormone with gonadotropin-releasing hormone analog after ovarian stimulation for in vitro fertilization. Fertil. Steril. 55, 328—332.

Itskovitz, J., Boldes, R., Levron, J., Erlik, Y., Kahana, L., Brandes, J.M., 1991. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil. Steril. 56, 213—220.

Jones Jr., H.W., 1996. What has happened? Where are we? Hum. Reprod. 11, 7—24 (discussion 29—31).

Kol, S., 2004. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fertil. Steril. 81, 1—5.

Kol, S., Itskovitz-Eldor, J., 2000. Severe OHSS: yes, there is a strategy to prevent it! Hum. Reprod. 15, 2266—2267.

Kolibianakis, E.M., Devroey, P., 2002. The luteal phase after ovarian stimulation. Reprod. Biomed. Online 5, 26—35.

Kolibianakis, E., Bourgain, C., Albano, C., Osmanagaoglu, K., Smitz, J., Van Steirteghem, A., Devroey, P., 2002. 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. 78, 1025—1029.

Kolibianakis, E.M., Schultze-Mosgau, A., Schroer, A., van Steirteghem, A., Devroey, P., Diedrich, K., Griesinger, G., 2005. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum. Reprod. 20, 2887—2892.

Kolibianakis, E.M., Collins, J., Tarlatzis, B.C., Devroey, P., Diedrich, K., Griesinger, G., 2006. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. Hum. Reprod. Update 12, 651—671.

Licht, P., Russu, V., Lehmeyer, S., Wildt, L., 2001. Molecular aspects of direct LH/HCG effects on human endometrium — lessons from intrauterine microdialysis in the human female in vivo. Reprod. Biol. 1, 10—19.

Macklon, N.S., Stouffer, R.L., Giudice, L.C., Fauser, B.C., 2006. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr. Rev. 27, 170—207.

Martel, D., Frydman, R., Glissant, M., Maggioni, C., Roche, D., Psychoyos, A., 1987. Scanning electron microscopy of postovulatory human endometrium in spontaneous cycles and cycles stimulated by hormone treatment. J. Endocrinol. 114, 319—324.

Maruncic, M., Casper, R.F., 1987. The effect of luteal phase estrogen antagonism on luteinizing hormone pulsatility and luteal function in women. J. Clin. Endocrinol. Metab. 64, 148—152.

Noyes, R.W., Hertig, A.T., Rock, J., 1975. Dating the endometrial biopsy. Am. J. Obstet. Gynecol. 122, 262—263.

Oktay, K., Turkcuoglu, I., Rodriguez-Wallberg, K.A., 2010. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod. Biomed. Online 20, 783—788.

Orvieto, R., 2005. Can we eliminate severe ovarian hyperstimulation syndrome? Hum. Reprod. 20, 320—322.

Rao, C.V., 2001. Multiple novel roles of luteinizing hormone. Fertil. Steril. 76, 1097—1100.

Shalev, E., Geslevich, Y., Matilsky, M., Ben-Ami, M., 1995. Gonadotrophin-releasing hormone agonist compared with human chorionic gonadotrophin for ovulation induction after clomiphene citrate treatment. Hum. Reprod. 10, 2541—2544.

Sharma, V., Whitehead, M., Mason, B., Pryse-Davies, J., Ryder, T., Dowsett, M., Campbell, S., Collins, W., 1990. Influence of superovulation on endometrial and embryonic development. Fertil. Steril. 53, 822—829.

Smitz, J., Bourgain, C., Devroey, P., Camus, M., Wisanto, A., Van Stierteghem, A.C., 1990. The luteal phase after GnRH analogues-HMG or clomiphene-HMG superovulation: endocrinology and endometrial morphology. In: Alsina, J.C. (Ed.), GnRH y análogos en medicina reproductiva. ESPAXS, Barcelona.

Sugino, N., Kashida, S., Takiguchi, S., Karube, A., Kato, H., 2000. Expression of vascular endothelial growth factor and its receptors in the human corpus luteum during the menstrual cycle and in early pregnancy. J. Clin. Endocrinol. Metab. 85, 3919—3924.

Tavaniotou, A., Albano, C., Smitz, J., Devroey, P., 2002. Effect of clomiphene citrate on follicular and luteal phase luteinizing hormone concentrations in in vitro fertilization cycles stimulated with gonadotropins and gonadotropin-releasing hormone antagonist. Fertil. Steril. 77, 733—737.

Teramoto, S., Kato, O., 2007. Minimal ovarian stimulation with clomiphene citrate: a large-scale retrospective study. Reprod. Biomed. Online 15, 134—148.

Tesarik, J., Hazout, A., Mendoza, C., 2003. Luteinizing hormone affects uterine receptivity independently of ovarian function. Reprod. Biomed. Online 7, 59—64.

Wang, T.H., Horng, S.G., Chang, C.L., Wu, H.M., Tsai, Y.J., Wang, H.S., Soong, Y.K., 2002. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. J. Clin. Endocrinol. Metab. 87, 3300—3308.

Whelan 3rd., J.G., Vlahos, N.F., 2000. The ovarian hyperstimulation syndrome. Fertil. Steril. 73, 883—896.

Yding Andersen, C., Leonardsen, L., Ulloa-Aguirre, A., Barrios-De-Tomasi, J., Moore, L., Byskov, A.G., 1999. FSH-induced resumption of meiosis in mouse oocytes: effect of different isoforms. Mol. Hum. Reprod. 5, 726—731.

Zelinski-Wooten, M.B., Hutchison, J.S., Hess, D.L., Wolf, D.P., Stouffer, R.L., 1995. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. Hum. Reprod. 10, 1658—1666.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 14 May 2013; refereed 15 October 2013; accepted 22 October 2013.