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Spontaneous triggering of ovulation versus HCG administration in patients undergoing IUI: a prospective randomized study

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Dr Kyrou received her medical degree (1997) from the University of Liege in Belgium and graduated as an obstetrician gynaecologist in 2007. After 3 years of training as a resident in infertility and endoscopic surgery in CRG, UZBrussel, she became specialist in reproductive medicine. Since October 2010, she has been the Scientific Director of the assisted reproduction unit BIOGENESIS, Thessaloniki, Greece. In 2011, she obtained her PhD under the supervision of Professor B Tarlatzis and Professor P Devroey at the Brussels Free University. Her current clinical and research interests include endometrial receptivity and reproductive endocrinology.

Abstract The objective of this prospective randomized study was to assess whether spontaneous triggering of ovulation by detecting LH rise with serial serum testing, results in higher pregnancy rates as compared with administration of human chorionic gonadotrophin (HCG) in patients undergoing intrauterine insemination (IUI) in natural cycles. The trial was registered in clinicaltrials.gov as NCT01414673. Three hundred patients treated by IUI in natural cycles at the Centre of Reproductive Medicine of the Dutch-Speaking Brussels Free University were randomized to either spontaneous triggering of ovulation (spontaneous LH group) ($n = 150$) or administration of HCG ($n = 150$). Donor spermatozoa was used in 197/300 patients (65.67%). The duration of the follicular phase was significantly higher in the spontaneous LH group as compared with the HCG group ($P = 0.004$). However, the ongoing pregnancy rate was significantly higher in the spontaneous LH group as compared with the HCG group (34/150 versus 16/150, $P = 0.008$; difference 12.0%, 95% CI − 3.6 to 20.4). The use of LH for timing ovulation in natural cycles might be the best way to maximize the probability of pregnancy for patients undergoing IUI.

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Introduction

Intrauterine insemination (IUI) involves timed insemination of spermatozoa into the uterus in natural cycles or insemination following stimulation of the ovaries using clomiphene citrate or gonadotrophins (Cantineau et al., 2007; Cohlen, 2005). Although IUI is less invasive and less expensive than IVF, IUI combined with ovarian stimulation increases the risk of multiple pregnancy compared with IUI in natural cycles (Fauser et al., 2005). The reported pregnancy rates per cycle for IUI in natural cycles ranges from 5.0% to 9.3% (Guzick et al., 1999; Martinez et al., 1990; Steures et al., 2007).

In an IUI cycle, spermatozoa and oocytes survive for a limited period of time. Thus, IUI should be performed as close to ovulation as possible (Barrett and Marshall, 1969; Royston, 1982; Schwartz et al., 1980). Timing of IUI is usually achieved by detecting LH rise or by administering human chorionic gonadotrophin (HCG). The follicular size at which LH rise might occur varies, as does the time interval between the onset of LH rise and ovulation (24–56 h) (World Health Organization, 1980). On the other hand, in the presence of a mature follicle, administration of HCG will lead to follicular rupture within 36–48 h.

Administration of HCG is a popular method for triggering ovulation, since it may avoid the need to perform IUI at weekends. However, it is an intervention compared with the timing of IUI by detection of the spontaneous LH rise. Moreover, due to the presence of HCG receptors in the human endometrium, administration of HCG might interfere with endometrial receptivity (Licht et al., 2007).

Currently, it remains unclear whether the probability of pregnancy is associated with the mode of ovulation triggering in IUI natural cycles. The aim of this study was to assess prospectively whether spontaneous triggering of ovulation by detecting LH rise results in higher pregnancy rates as compared with administration of HCG in patients undergoing IUI.

Materials and methods

Patients treated by IUI in natural cycles at the Centre of Reproductive Medicine of the Dutch-Speaking Brussels Free University were randomized at the outpatient clinic by the treating physician on the basis of a computer-generated list to either spontaneous triggering of ovulation (spontaneous LH group) or administration of HCG (HCG group).

Patients could participate in the study only once. Inclusion criteria were: age ≤36 years, regular menstrual cycles, body mass index (BMI) between 18 and 29 kg/m^2, basal concentrations of FSH (≤12 IU/l), oestradiol (≤80 pg/ml) and progesterone (≤1.6 ng/ml) on day 1 of the cycle and normal hysterosalpingography (maximum 3 months to prior starting the cycle). Couples with male factor were included when semen had more than 5 million motile spermatozoa/ejaculation in the initial evaluation (Khalil et al., 2001) and normal morphology >4% (Van Waart et al., 2001). The use of donor spermatozoa was also accepted as inclusion criteria. Exclusion criteria were the presence of polycystic ovarian syndrome (Rotterdam criteria) and endometriosis (≥AFS III).

The Institutional Review Board approved the research project and informed consent was obtained from all patients participating in the study (IRB reference: B.U.N.B14320096060/2009). The trial was registered in clinicaltrials.gov as NCT01414673.

On day 1 of the treatment cycle, patients underwent a blood test to confirm normal values of oestradiol, progesterone and FSH. In the spontaneous LH group, daily monitoring of LH with serial serum testing could start from day 6 of the cycle (if necessary) until LH rise. In the HCG group, as soon as a follicle reached a diameter of ≥17 mm, 5000 IU of HCG (Pregnyl; Merck Sharpe Dome) was administered. When LH started to rise, a second assessment was performed on the following day to confirm the LH rise. In the natural cycle, criteria for detection of initiation of the LH surge included an LH rise of 180% above the latest serum value available in that patient that continued to rise in successive assessments (Testart et al., 1981). A single insemination was performed 36 h post HCG or post initiation of the LH rise using a Friedman catheter. A limited insemination volume (0.3 ml) was delivered into the uterine cavity and bed rest was maintained for 10 min after the procedure. In case the first assessment of serum LH suggested an imminent ovulation, defined as LH surge (LH rise accompanied by serum progesterone concentration of ≥1.6 ng/ml), the insemination was performed 24 h later. Therefore, taking into account the detection or not of an LH surge in both groups, IUI was performed 24 h or 36 h later in all patients. Inseminations were performed 7 days a week in the study centre. Serum HCG test was performed 14 days after insemination.

Ultrasound and laboratory assays

Ultrasound was performed on day 6 of the cycle and thereafter as necessary in order to ensure that HCG would be injected on the first day that the patient had one follicle of ≥17 mm (HCG group). For that purpose, a follicular growth of 2 mm per day was assumed.

Ultrasound measurements were performed by four observers who also performed the ultrasounds of patients not included on the study using the same method. The ultrasound image was frozen when the follicle appeared maximal and two dimensions perpendicular to each other were measured, from which the average follicular diameter was calculated. In the presence of a positive HCG test, an intrauterine gestational sac was confirmed at ultrasound at 7 weeks of gestation. An ultrasound at 12 weeks of gestation was performed to confirm the presence of ongoing pregnancy.

Serum LH, FSH, oestradiol, progesterone and HCG were measured by means of the automated Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation (CVs) were <3% and <4% for LH, <3% and <6% for FSH, < 5% and <10% for oestradiol, <3% and 5% for progesterone and <5% and <7% for HCG, respectively.

Outcome measure

The outcome measure was detection of ongoing pregnancy defined as pregnancy progressing beyond 12 weeks of gestation.

Statistics

Currently, as far as is known, there are no published studies comparing HCG administration to detection of spontaneous LH rise for timing IUI during a natural cycle. Based on the meta-analysis by Kosmas et al. (2007) that compared HCG versus detection of spontaneous LH rise in patients treated with clomiphene citrate, a difference of 3% in favour of spontaneous LH group was detected. It was calculated that a sample size of 2943 patients was required in each group to achieve 80% power at a 5% significance level to detect a difference of 3%, which was considered as clinically important, assuming a 23% ongoing pregnancy rate in the spontaneous LH group and a 20% ongoing pregnancy rate in the HCG group. Obtaining such a sample size is not easy to achieve in a single centre; thus, the aim of the current study was to assess whether spontaneous triggering of ovulation by detecting LH rise results in higher pregnancy rates as compared with administration of HCG in patients undergoing IUI on a relatively large patient population. The results of this study could be included in a future meta-analysis on this issue.

Continuous variables were compared with the t-test for independent samples or the Mann–Whitney U test, depending on the normality of their distribution. Proportions were compared with the Fisher's exact test or the chi-squared test where appropriate. $P < 0.05$ was considered statistically significant. A subgroup analysis was performed comparing ongoing pregnancy rate in both groups according the presence or not of an LH surge.

Results

Three hundred patients treated by IUI from April 2009 until October 2010 were included in the study. The flowchart of the patients is shown in Figure 1.

Table 1 describes the baseline characteristics and hormonal profile of patients in the HCG and spontaneous LH group. No significant differences were observed regarding age, BMI, number of previous attempts, indications for treatment and basal FSH between patients in the two groups compared. Similarly, no significant differences were observed regarding endometrial thickness and progesterone concentration on the day of HCG administration or the day of detecting a spontaneous LH rise. The proportion of patients in whom donor spermatozoa were used was similar between the groups (70.0% versus 61.3%, respectively).

Estradiol and LH concentrations on the day of HCG administration or the day of detecting spontaneous LH rise were significantly higher in the spontaneous LH versus the HCG group ($P = 0.01$ and $P = 0.001$ respectively). The duration of the follicular phase was significantly longer in the spontaneous LH group as compared with the HCG group ($P = 0.004$; Table 1).

The ongoing pregnancy rate was significantly higher in the spontaneous LH group as compared with the HCG group (22.7% versus 10.7%; $P = 0.008$; Table 2). Additionally, a significant difference was observed in favour of the spontaneous LH group when the indication for treatment was female infertility (21/87, 24.1% versus 10/88, 11.4%; $P = 0.03$).

A subgroup analysis was performed taking into account the detection or not of an LH surge in both groups. In the
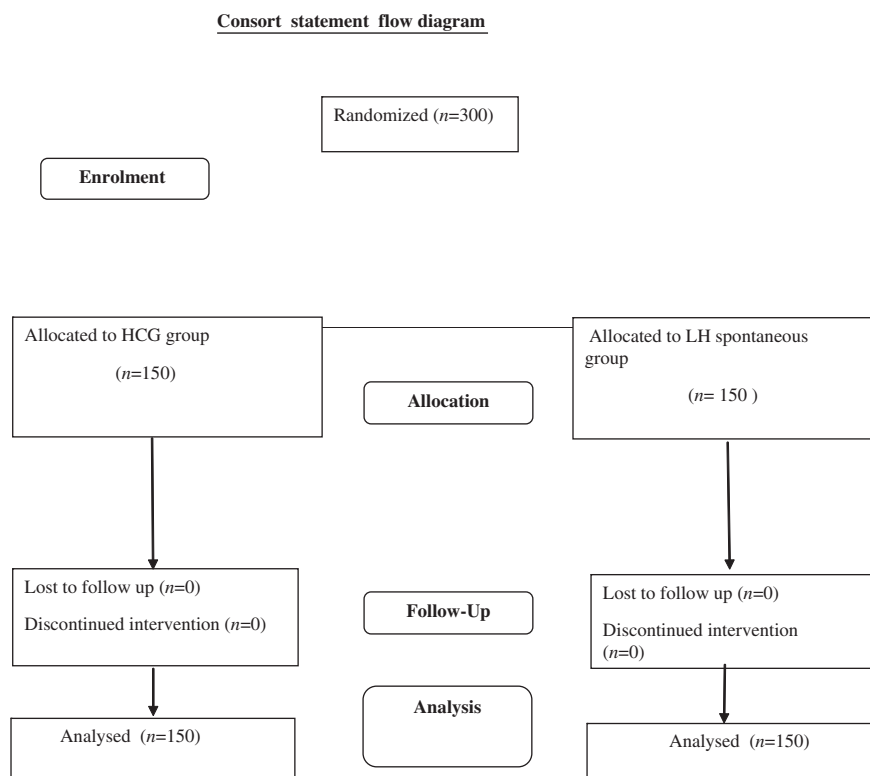

Figure 1 Patient flow chart. HCG = human chorionic gonadotrophin.

Table 1 Comparison of patient characteristics and stimulation data according to treatment group.

	HCG administration (n = 150)	Spontaneous LH rise (n = 150)	P-value
Age (years)	31.37 ± 3.71	31.49 ± 3.72	NS
Body mass index (kg/m^2)	23.40 ± 3.08	23.00 ± 2.63	NS
No. of IUI cycles (including the studied cycle)	1.95 ± 0.44	1.75 ± 0.88	NS
Indications of treatment			NS
Male	37 (24.7)	24 (16.0)	
Idiopathic	25 (16.7)	39 (26.0)	
Lesbian	57 (38.0)	53 (35.3)	
Single mother	31 (20.7)	34 (22.7)	
Type of spermatozoa			NS
Partner	45 (30.0)	58 (38.7)	
Donor	105 (70.0)	92 (61.3)	
Basal FSH (IU/l)	6.94 ± 1.52	6.66 ± 1.57	NS
Length of follicular phase (days)	12.15 ± 2.40	14.33 ± 3.81	0.004
Hormone concentrations on day of HCG or spontaneous LH rise			
Estradiol (pg/ml)	112.53 ± 169.89	326.38 ± 283.22	0.010
Progesterone (ng/ml)	0.75 ± 0.80	0.82 ± 0.43	NS
LH (mIU/ml)	13.53 ± 9.05	35.62 ± 21.44	0.001
Endometrial thickness (mm)	8.12 ± 1.87	8.35 ± 1.74	NS

Values are mean ± SD or *n* (%).
HCG = human chorionic gonadotrophin; IUI = intrauterine insemination; NS = not statistically significant.

Table 2 Treatment outcomes between the study groups.

	HCG administration	Spontaneous LH rise	Difference (%, 95% CI)	P-value
Ongoing pregnancy	16/150 (10.7)	34/150 (22.7)	−12.0 (−20.4 to −3.6)	0.008
Ongoing pregnancy with LH surge detected (IUI 24 h)	5/22 (22.7)	17/72 (23.6)	−0.9 (−21.4 to 17.6)	NS
Ongoing pregnancy with no LH surge detected (IUI 36 h)[a]	11/128 (8.6)	17/78 (21.8)	−13.2 (−24.2 to −3.3)	0.011

Values are *n*/total (%) unless otherwise stated.
IUI = intrauterine insemination; NS = not statistically significant.
[a]IUI 36 h after LH rise in spontaneous LH group.

presence of an LH surge, IUI was performed after 24 h instead of 36 h in all patients (94/300). No difference in ongoing pregnancy rates was observed between the two groups when LH surge was detected and IUI was performed 24 h later. On the contrary, a significantly lower ongoing pregnancy rate was observed in the HCG group compared with the spontaneous LH group, when no LH surge was detected and IUI was performed in 36 h after the administration of HCG or detection of LH rise (*P* = 0.011; **Table 2**).

Discussion

As far as is known, this is the first randomized controlled trial examining whether spontaneous triggering of ovulation by detecting LH rise results in higher ongoing pregnancy rates as compared with administration of HCG in patients undergoing IUI. A significantly higher ongoing pregnancy rate was observed in the spontaneous LH group compared with the HCG group.

The lower pregnancy rate in the HCG group was not observed when an LH surge was detected and IUI was performed the following day. In this case, ongoing pregnancy rates were similar to those observed in the spontaneous LH group when LH surge was detected and IUI was brought forward by 1 day (**Table 2**).

In a meta-analysis, including both prospective and retrospective studies that compared spontaneous LH rise versus HCG administration for triggering ovulation in patients stimulated with clomiphene citrate, a significantly higher pregnancy rate was observed in favour of the spontaneous LH rise group (Kosmas et al., 2007). However, in a subsequent

meta-analysis including only prospective studies and comparing spontaneous LH rise versus HCG administration for triggering ovulation in patients stimulated with clomiphene citrate or human menopausal gonadotrophin, no significant difference was observed between the two methods (Cantineau et al., 2010).

Evidence for the superiority of the natural cycle in which triggering of ovulation occurs by the spontaneous LH rise as compared with a natural cycle in which triggering of ovulation is controlled by HCG administration is present in frozen—thawed embryo transfer cycles (Fatemi et al., 2010).

One of the questions arising from the results obtained in the current study is whether the significant differences in ongoing pregnancy rate in spontaneous LH surge versus HCG triggering are related to the timing of fertilization and blastocyst arrival to the endometrium, the changes induced by LH in the endometrium during a non-stimulated natural cycle or both. It has been reported that endometrial receptivity is higher in non-stimulated compared with stimulated IVF cycles. Since the window of implantation has been reported to span from 4 to 5 days, it is more likely that the observed differences in pregnancy rate are related to specific LH-induced changes in the endometrium that favour embryo implantation.

The timing of events that lead to endometrial receptivity might be associated with the type of signal triggering ovulation. Apparently, in the presence of the LH surge, where IUI is performed 24 h later, the cascade of events leading to ovulation and a receptive endometrium is initiated naturally and driven primarily by the LH surge itself and to a lesser extent by exogenous HCG. This might be the explanation for the lack of difference in ongoing pregnancy rates when the LH surge was detected which occurred in approximately one-third of patients.

The higher ongoing pregnancy rate in the spontaneous cycles might also be associated with the degree of follicle/oocyte maturity during the LH rise compared with the case of HCG administration. Clinical experiments have shown that in hypophysectomized subjects, a rise of plasma oestradiol to 200 pg/ml sustained for 50 h was necessary to trigger the LH rise. This old finding could explain the fact that the follicular phase was longer and that the success rate was higher in the spontaneous group. If HCG is triggered prematurely, the oocyte will probably be immature and pregnancy will not occur (Young and Jaffe, 1976).

The criterion for HCG administration was arbitrarily decided to be the presence of a follicle of $\geq$17 mm. It could be argued that the results obtained might not be valid if different criteria were used for HCG administration; however, no universally accepted criteria for this purpose exist (Kolibianakis et al., 2004). The same applies to the use of a urinary ovulation predictor kit instead of serial serum LH testing for the determination of the LH rise.

It has to be considered that detection of LH for triggering ovulation might not be applicable in centres not working on weekends. Moreover, it is associated with an increase in the length of the follicular phase and the number of clinic visits. On the other hand, the above shortcomings have to be balanced against an increased probability of pregnancy in the spontaneous LH group.

Additionally, it might be argued, that the efficacy in terms of pregnancy outcome of spontaneous triggering

based on LH rise in IUI natural cycles in couples with the inclusion criteria described in this study is comparable or even higher than that (12%) reported for FSH-stimulated cycles and triggering of ovulation with HCG (Cantineau et al., 2010). Thus, since IUI with spontaneous LH rise triggering is significantly less expensive and the risks of ovarian hyperstimulation and multiple pregnancy are negligible compared with IUI with FSH stimulation, comparative studies between FSH-stimulated cycles and spontaneous LH rise triggering in natural cycles would be worthwhile.

The high pregnancy rate observed in this study could be easily explained by the fact that a large number of the treated subjects were lesbians and single mothers, who used donor spermatozoa.

Future studies, besides aiming to confirm the findings of the current trial, should further focus on endometrial alterations at the histological as well as the gene expression level in natural cycles triggered by either spontaneous LH rise or HCG administration (Licht et al., 2001, 2007; Fluhr et al., 2008).

In conclusion, spontaneous triggering of ovulation is associated with significantly higher ongoing pregnancy rates compared with administration of HCG in patients undergoing IUI. Therefore, the use of LH for timing ovulation in natural cycles might be the best way to maximize the probability of pregnancy for patients undergoing IUI.

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