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ORIGINAL ARTICLE ***Infertility***

Should an intrauterine insemination with donor semen be performed 1 or 2 days after the spontaneous LH rise? A prospective RCT

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STUDY QUESTION: What is the impact on pregnancy rates when intrauterine insemination (IUI) is performed 1 or 2 days after the spontaneous LH rise?

SUMMARY ANSWER: IUI 1 day after the spontaneous LH rise results in significantly higher clinical pregnancy rates compared with IUI performed 2 days after the LH rise.

WHAT IS KNOWN ALREADY: IUI is scheduled within a limited time interval during which successful conception can be expected. Data about the optimal timing of IUI are based on inseminations following ovarian stimulation. There is no available evidence regarding the correct timing of IUI in a natural menstrual cycle following the occurrence of a spontaneous LH rise.

STUDY DESIGN, SIZE, DURATION: A prospective RCT, including patients undergoing IUI with donor sperm in a natural menstrual cycle. IUI cycles ($n = 435$) were randomized between October 2010 and April 2013, of which 23 were excluded owing to protocol deviation and 412 received the allocated intervention.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Serial serum LH concentrations were analysed in samples taken between 07:00 and 09:00 h to detect an LH rise from Day 11 of the cycle onwards. The subjects were randomized to receive insemination either 1 or 2 days after the observed LH rise. In the final analysis, there were 213 cycles in the group receiving IUI 1 day after the LH rise and 199 cycles in the group receiving IUI 2 days after the LH rise.

MAIN RESULTS AND THE ROLE OF CHANCE: Significantly higher clinical pregnancy rates per IUI cycle were observed in patients undergoing IUI 1 day after the LH rise when compared with patients undergoing IUI 2 days after the LH rise [19.7 (42/213) versus 11.1% (22/199), $P = 0.02$]. In view of the timing of sampling for LH, the inseminations were performed at 27 h (± 2 h) and 51 h (± 2 h) after detection of the LH rise. The risk ratio of achieving a clinical pregnancy if IUI was scheduled 1 day after the LH rise compared with 2 days was 1.78 [95% confidence interval (CI), 1.11–2.88]. This points towards a gain of one additional clinical pregnancy for every 12 cycles performed 1 day instead of 2 days after the LH rise. When analysing the results per patient, including only women who underwent their first treatment cycle of insemination, the outcome was in line with the per cycle analysis, demonstrating an 8% difference in pregnancy rate in favour of the early group (20.5 versus 12.2%), however, this difference was not significant.

LIMITATIONS, REASONS FOR CAUTION: Optimal monitoring for the occurrence of the LH rise involves several daily LH measurements, which is not always amenable to everyday clinical practice, however, daily sampling was sufficient to detect a significant difference in pregnancy rate. The strict inclusion of a highly selected population of patients who underwent IUI in a natural cycle may have been a limitation. IUI in a natural menstrual cycle confers lower success rates compared with IUI following ovarian stimulation and is not suitable for patients with ovulatory dysfunction. Furthermore, a similar study in a larger number of women is required to confirm the result in terms of pregnancy rate per patient.

WIDER IMPLICATIONS OF THE FINDINGS: This is the first RCT to show that timing of IUI in a natural menstrual cycle is important and that IUI should be performed 1 day after the LH rise, rather than 2 days post-LH rise. Daily monitoring of the rise in LH, as performed in our study, can be adopted to achieve a higher pregnancy rate per IUI cycle.

STUDY FUNDING/COMPETING INTEREST(S): No funding was received for this study. All authors declare to have no conflict of interest with regard to this trial.

TRIAL REGISTRATION NUMBER: The trial was registered at clinicaltrials.gov (NCT01622023).

Key words: intrauterine insemination / LH rise / timing / natural cycle

Introduction

Intrauterine insemination (IUI) is a widely used method for a broad range of indications in reproductive medicine. It is commonly applied to couples diagnosed with mild male factor infertility, endometriosis or unexplained infertility (ESHRE Capri Workshop, 2009). Insemination with donor sperm has an important role in the treatment of severe male infertility, and is often used in lesbian couples and single parents (National Institute for Health and Clinical Excellence, 2013). According to the latest European IVF-Monitoring programme, 24 960 donor sperm IUIs were performed in 21 reporting countries in 2008 (Ferrareti *et al.*, 2012). The method involves the timely scheduled transfer of spermatozoa into the uterus, either in a natural menstrual cycle or following ovarian stimulation with anti-estrogens or gonadotrophins (ESHRE Capri Workshop, 2009). Although IUI following ovarian stimulation results in higher pregnancy rates and requires less endocrine monitoring compared with IUI in a natural menstrual cycle, the latter approach confers in a benefit of a lower medication cost and a lower prevalence of multiple gestations (Fauser *et al.*, 2005; Steures *et al.*, 2007). From this perspective, IUI in a natural menstrual cycle is safer for the patient and merits consideration.

Correct scheduling is paramount to success of IUI, because of the limited time interval in which capacitated spermatozoa survive in the female genital tract and oocytes can be fertilized after ovulation. However, robust evidence regarding appropriate scheduling of IUI in a natural cycle is lacking and the scarce available data are inconclusive (Ragni *et al.*, 2004). Spermatozoa can retain their capability to fertilize the oocyte and survive in the female reproductive tract for ~3 days after ejaculation, while oocytes remain fertilizable only for 12–16 h after ovulation (Edwards and Steptoe, 1974; Gould *et al.*, 1984). However, the above holds true for spermatozoa that have actively travelled through the cervix *in vivo*. In the case of IUI, however, spermatozoa are processed, devoid of seminal plasma and only the fraction of selected, motile spermatozoa is injected directly into the uterine cavity. Subsequently, they can migrate through the tubal ostia into the abdominal cavity, where they have been observed through laparoscopy only a few hours after the insemination (Weathersbee *et al.*, 1984; Ripps *et al.*, 1994). Considering these facts, the time frame within which a successful conception after IUI is possible is substantially shorter than in the setting of natural conception. Hence, the procedure should ideally be scheduled as closely to the time of ovulation as possible to increase the probability of fertilization (Cantineau *et al.*, 2010).

When considering the scheduling of IUI, two approaches are generally accepted, i.e. administration of hCG for final oocyte maturation or, alternatively, the identification of the spontaneous LH rise. When hCG is administered, ovulation usually occurs 36–42 h later, and IUI is most commonly performed 32–36 h after hCG administration (Edwards and Steptoe, 1974; Andersen *et al.*, 1995). On the other hand, ovulation occurs 24–56 h after the onset of the spontaneous LH rise, with an average of 32 h (WHO, 1980; Robb *et al.*, 2004). The variable time frame during which ovulation takes place, combined with the requirement of close monitoring to detect the spontaneous LH rise, makes correct scheduling of IUI based on the detection of the spontaneous LH rise a challenging procedure.

However, published evidence suggests that the success rate of IUI in a natural cycle is higher when IUI is scheduled after the spontaneous LH rise compared with IUI after hCG administration (Kyrou *et al.*, 2012). Other published studies comparing IUI success rates after the spontaneous LH rise and IUI after hCG administration have been performed after ovarian stimulation (Kosmas *et al.*, 2007; Cantineau *et al.*, 2010). When ovulation is triggered using hCG administration compared with spontaneous ovulation, as detected by monitoring of serum LH levels in patients stimulated with clomiphene citrate undergoing IUI, higher pregnancy rates can be expected after the spontaneous LH rise, as demonstrated by a systematic review including seven prospective and retrospective studies comprising 2481 patients in total (Kosmas *et al.*, 2007). This finding has not been confirmed in a subsequent systematic review including only the prospective studies, with 275 patients (Cantineau *et al.*, 2010). The goal of our trial was to prospectively determine the most appropriate timing of IUI after the spontaneous LH rise in a natural menstrual cycle, comparing clinical outcomes in patients who underwent IUI either 1 day or 2 days after detection of the LH rise.

Materials and Methods

Study design

In this prospective RCT, patients undergoing IUI with donor sperm in a natural menstrual cycle were considered eligible. The trial was set up to compare two scheduling options for IUI and was conducted at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University between October 2010 and May 2013. The Institutional Review Board approved the research project and informed consent was obtained from all patients participating to the study. The study was registered with the Clinical Trial website (www.clinicaltrials.gov, number NCT01622023).

Eligibility criteria

Women who were eligible for the RCT were between 18 and 39 years old, with regular menstrual cycles (21–35 days) and had previously undergone no more than six unsuccessful IUI attempts.

Exclusion criteria were the presence of tubal factor infertility or endometriosis classified as American Fertility Society III or higher. Patients who had a serum progesterone level above 1.2 ng/ml on the day of the LH rise and

patients in whom three or more follicles ≥15 mm were observed on the day of LH rise were also excluded from the study. Indications for the use of donor sperm were male factor infertility due to azoospermia or severe oligoasthenoteratozoospermia (requiring donor sperm), the presence of a heritable genetic disorder in the male partner, a single-parent request or lesbian couples' partners undergoing IUI.

The randomization procedure took place during the daily monitoring sessions at the clinic, when an LH rise was observed. The randomization was performed on the IUI cycle level as reported previously (Vermeylen *et al.*, 2006). Randomization into one of the two groups was performed using an open computer-generated list.

Patient monitoring and IUI treatment

In the early follicular phase (Day 2 or 3) of the menstrual cycle, all patients underwent serum analysis of FSH, LH, estradiol (E_2) and progesterone levels, to confirm basal serum levels. Blood sampling was performed in the morning, from 7 a.m. to 9 a.m. Further blood sampling in combination with pelvic ultrasound scanning was scheduled on Day 11 of the menstrual cycle. Afterwards, serial serum hormone monitoring was carried out daily or every other day according to the hormone analysis and ultrasound results. IUI was performed either 1 day or 2 days after the detection of the LH rise, according to the study group. IUI was performed with a Frydman catheter depositing 0.2–0.5 ml of the sperm sample at the uterine fundus. All IUIs were performed between 10 a.m. and 12 p.m. After the procedure, 10 min of bed rest were prescribed to the patients (Custers *et al.*, 2009). Inseminations were performed every day of the week, including weekends. To assess the treatment outcome, serum hCG was measured 14 and 17 days after IUI. HCG levels above 20 IU/l indicated pregnancy. Clinical pregnancy was defined as the observation of fetal cardiac activity on ultrasonography at 7 weeks of gestation.

Serum LH monitoring was performed using the Elecsys (Roche Diagnostics, Germany) immunoassay LH kit. The analytical sensitivity of the kit is 0.10 mIU/ml at a total imprecision (% coefficient of variation) of <6. An LH rise was defined as an increment of 80% compared with the previous serum LH level available from a given patient, to a level above 18 IU/l (Testart *et al.*, 1981). This arbitrary cut-off of 18 IU/l was chosen in order to achieve a low false-positive rate of the detected LH rise.

Outcome measures

The primary end-point of the trial was the clinical pregnancy rate per IUI cycle in both treatment groups. Secondary end-points included the rate of positive hCG results and biochemical pregnancies in both groups as well as the endocrine profile of the included subjects on the day of the LH rise. Demographic and clinical characteristics, including age, BMI and baseline hormone levels, were also analysed.

Statistical analysis

According to our sample size calculation, 398 IUI cycles in total (199 in each arm) were essential, in order to detect an increase in clinical pregnancy rate from 10 to 20% between both groups with a power of 80% and two-sided 5% significance level. With a predicted drop-out level of 5%, we aimed to recruit 420 cases. To recruit this number of IUI cycles, a 2-year inclusion period was anticipated.

Mean values and SD were calculated for each continuous variable and percentages for the categorical variables. The comparison between groups was then presented as a relative effect [risk ratio with a corresponding 95% confidence interval (CI)] as well as the absolute effect (risk difference with a corresponding 95% CI). We also used independent samples *t*-test or Mann–Whitney *U*-test to compare continuous variables, depending on the normality of the distribution. Fisher's exact *P*-value test was used to compare categorical variables. Owing to a statistically significant difference between groups in one of the confounding factors (BMI), a multivariable logistic regression model was constructed in the sensitivity analysis. For all tests, we assumed a two-sided *P*-value of 0.05 as an indication of statistical significance. Statistical analyses were performed using the Statistical Package for the Social Sciences 20.0 software (SPSS Inc., Chicago, IL, USA).

Results

A total number of 435 IUI cycles were recruited during the study period. They were randomized to undergo IUI either 1 or 2 days after LH rise detection. The trial profile is depicted in Fig. 1. Owing to a deviation from the study protocol, 10 cycles were excluded in the group of patients undergoing IUI 1 day after the LH rise, as well as 13 cycles in the group undergoing IUI 2 days later. Ultimately, 304 women undergoing 412 IUI cycles were included in the final analysis (Fig. 1). Among these patients, 211 women were included in a single treatment cycle, whereas 93 women underwent more than one treatment cycle.

The baseline demographic characteristics were comparable between both groups as demonstrated in Table I. The patients did not differ in mean age or the number of previous IUI attempts or the number of previously achieved pregnancies and live births (Table I). None of the patients underwent more than five previous IUI attempts. Only their BMI was statistically different ($P = 0.004$), conferring a mean difference of 1.3 (0.4) kg/m^2, and should therefore not to be considered as relevant. Baseline serum levels of FSH, LH, E_2 and progesterone were comparable in both treatment arms (Table II).

When analysing serum hormone levels on the day of the LH rise detection, the mean LH and progesterone levels were comparable between both groups of patients (Table II).

The clinical pregnancy rate was significantly higher when IUI was performed 1 day versus 2 days after the LH rise (19.7 versus 11.1%, respectively, $P = 0.02$), with an absolute difference of 8.7% (95% CI, 1.8–15.6%, Table III). For every 12 cycles performed, one additional clinical pregnancy could be expected.

These results were similar also when 'intention-to-treat' analysis, including all of the 435 initially recruited cycles was conducted. The pregnancy rate was significantly higher when IUI was performed on the day following detection of the LH rise when compared with 2 days later (19.4 versus 11.3%, respectively, $P = 0.02$). When analysis was restricted only to patients undergoing their first cycle of IUI, the difference in clinical pregnancy rates was 8% (20.5 versus 12.2% for Day 1 versus 2) and these results were not statistically significant ($P = 0.06$).

Owing to the significant difference in BMI of women between treatment arms, we constructed a multivariable logistic regression model in the sensitivity analysis, adjusting for this confounder. The adjusted odd ratio for achieving clinical pregnancy rate in the IUI group 1 day after the LH rise was 2.09 (95% CI, 1.17–3.73).

Discussion

The present study is the first RCT evaluating the impact of IUI scheduling on clinical success rates after a spontaneous LH rise in a natural menstrual cycle. Our data demonstrate that IUI 1 day after a spontaneous LH rise results in significantly higher clinical pregnancy rates compared with IUI 2 days after the detected LH rise.

Although the timing of IUI is physiologically important for eventual fertilization and thus pregnancy, limited data are available. To date, no

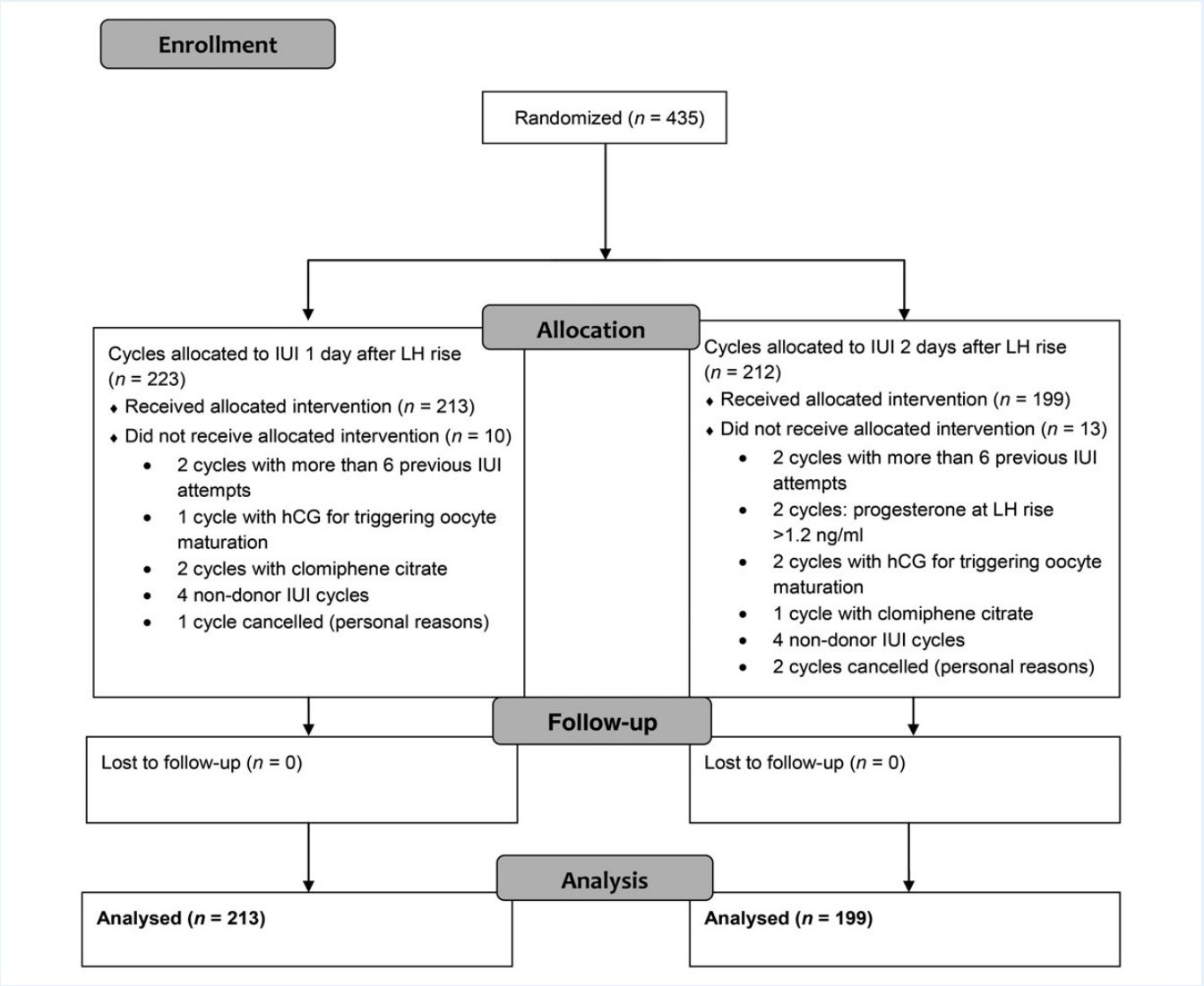

Figure 1 Flow chart for RCT of patients undergoing IUI in a natural cycle. IUI, intrauterine insemination.

studies have been performed investigating the influence of IUI scheduling on clinical outcomes in a natural menstrual cycle. All published data are based on cycles where hCG was administered for final oocyte maturation triggering. In view of this, a number of these studies have failed to demonstrate any significant influence of IUI timing on pregnancy rates (Huang *et al.*, 2000; Claman *et al.*, 2004; Robb *et al.*, 2004; Rahman *et al.*, 2011). However, there is a significant heterogeneity among the study designs, which precludes robust conclusions. More specifically, diverging time frames have been studied, including 24 versus 36 h after hCG (Robb *et al.*, 2004; Rahman *et al.*, 2011), 26–28 versus 36–38 h (Huang et al., 2000) or 33 versus 39 h after hCG (Claman *et al.*, 2004). In addition, different ovarian stimulation protocols were adopted with either clomiphene citrate (Robb *et al.*, 2004; Rahman *et al.*, 2011) or gonadotrophins (Huang et al., 2000; Claman *et al.*, 2004) and thus, no solid conclusion can be reached. Finally, although initial studies proposed double IUI within 36 h (Ragni *et al.*, 1999) as a strategy to overcome the problem of potentially inappropriate scheduling of IUI, the results of recent meta-analyses demonstrated that this approach does not improve the success rate of IUI (Cantineau *et al.*, 2003; Polyzos *et al.*, 2010; Zavos *et al.*, 2013).

The time of ovulation after a naturally occurring LH surge is subject to higher variability than after hCG administration and its prediction remains challenging. Available data show that ovulation can occur within a wide time frame, ranging from 24 to 56 h after the onset of the LH rise (WHO, 1980; Garcia *et al.*, 1981; Testart and Frydman, 1982; Robb *et al.*, 2004). This can be explained by the various definitions of the LH rise and the methods used for its detection (Fuh *et al.*, 1997). We adopted an approach based on a study by Testart *et al.* (1981) where LH levels were monitored several times daily to accurately detect the onset of LH rise. Nevertheless, such an approach is difficult to implement in daily clinical practice. The aim of our study was essentially to demonstrate if the scheduling of IUI could be optimized according to an approach adoptable in everyday clinical practice. As demonstrated by our results, this can be achieved by monitoring LH levels once daily

Table I Baseline demographic and clinical characteristics of patients in the RCT.

	IUI after 1 day ($n = 213$)	IUI after 2 days ($n = 199$)
Age (years), mean (SD)	32.0 (4.1)	32.1 (3.8)
Previous pregnancy (yes)	55 (25.8%)	62 (31.2%)
Previous live birth (yes)	28 (13.1%)	35 (17.7%)
Number of previous IUI cycles, median (IQR)	1 (0–3)	1 (0–3)
BMI, mean (SD)	23.8 (3.9)	25.1 (4.7)[a]
Indication for treatment		
Lesbian couple	129	121
Single women	40	36
Andrologic	44	42

IUI, intrauterine insemination.
[a]All parameters except BMI ($P = 0.004$) are not statistically significantly different between groups.

Table II Serum hormone levels on Day 2 of the natural menstrual cycle and on the day of LH rise.

	IUI after 1 day ($n = 213$)	IUI after 2 days ($n = 199$)	*P*
Day 2 of the menstrual cycle			
FSH (IU/l)	7.6 (3.0)	7.4 (2.1)	0.327
LH (IU/l)	6.0 (2.1)	5.8 (2.1)	0.325
Progesterone (ng/ml)	0.6 (0.3)	0.6 (0.3)	0.818
Estradiol (pg/ml)	46.2 (23.7)	43.7 (22.2)	0.278
Day of LH rise			
FSH (IU/l)	9.2 (4.4)	9.2 (3.9)	0.961
LH (IU/l)	33.7 (16.6)	32.8 (15.9)	0.567
Progesterone (ng/ml)	0.7 (0.3)	0.8 (0.2)	0.183
Estradiol (pg/ml)	296.9 (109.2)	276.6 (115.6)	0.067

IUI, intrauterine insemination.

when the follicle is reaching sonographic maturity. Caution should be taken when extrapolating our findings to the use of urinary LH kits for monitoring, as these assays can produce false-negative results in the lower ranges of LH values (<40 mIU/ml) and are generally accepted as less reliable compared with blood serum monitoring.

One limitation of our study is that the randomization was performed on the IUI cycle level. This means that the results are reported as the success rate per IUI cycle rather than per every treated patient. However, the per IUI cycle approach was employed in previous trials investigating the success of IUI (Vermeylen *et al.*, 2006). When analysing the results per patient, including only women who underwent their first treatment cycle of insemination, the outcome was in line with the per cycle analysis, demonstrating an 8% difference in pregnancy rate in favour of the early group (20.5 versus 12.2%). However, these results did not reach significance, probably due to the limited number of patients included in the trial. *Post hoc* power analysis demonstrated that in order to detect a difference of 8% with a two-sided Fisher's exact test, a power of 80% and a level of significance set at 0.05, a sample size of 700 patients would be required in a per patient analysis.

Furthermore, the IUIs were not performed exactly 24 and 48 h after the detected LH rise. Considering the timing of serum LH detection and IUI scheduling, it meant that the inseminations were performed at 27 h (±2 h) and 51 h (±2 h) after the LH rise detection. A further limitation is the strict inclusion of a highly selected population of patients, who underwent IUI in a natural cycle. IUI in a natural cycle yields lower pregnancy rates in patients with unexplained infertility compared with IUI following ovarian stimulation (Cohlen *et al.*, 2000; ESHRE Capri Workshop, 2009) and cannot be applied in women with anovulation—these patients should undergo induction of ovulation with clomiphene citrate or gonadotrophins (Homburg *et al.*, 2012).

The restriction of our study population to women requiring insemination with donor semen allowed the exclusion of potential confounding male or female fertility problems that could influence the outcome of IUI and would present a bias to the results and the conclusion. Hence, sperm quality, which has a significant impact on the success rate of IUI (Ombelet *et al.*, 2003; Wainer *et al.*, 2004), was not a confounding factor in our study. Nonetheless, the optimization of IUI scheduling should be equally important in patients who undergo IUI because of mild male factor infertility or other indications. Hence, we believe the results also apply to this group of patients.

In conclusion, our trial has shown for the first time that the IUI scheduling, with respect to time of the spontaneous LH rise, has a significant impact on clinical pregnancy rates when IUI is performed in a natural menstrual cycle. This is of paramount importance for clinics managing patients who undergo IUI in a natural menstrual cycle. IUI should be

Table III IUI outcome according to the timing (24 versus 48 h after the LH rise).

	IUI after 1 day ($n = 213$)	IUI after 2 days ($n = 199$)	Risk ratio (95% CI)	Risk difference (95% CI)
Positive hCG	49/213 (23.0%)	29/199 (14.6%)	1.58 (1.04–2.39)	8.4 (0.9–15.9)
Biochemical pregnancy	7/49 (14.5%)	7/29 (24.1%)	0.59 (0.23–1.52)	−9.8 (−28.3 to 8.5)
Clinical pregnancy	42/213 (19.7%)	22/199 (11.1%)	1.78 (1.11–2.88)	8.7 (1.8–15.6)

IUI, intrauterine insemination.

performed on the day following the LH rise and should not be postponed to the following day.

Authors' roles

C.B. made substantial contribution to the concept and design of the study. C.B., H.T., M.D.V., M.C. and N.P.P. contributed to acquisition of the data. J.K, C.B., N.P.P. contributed to the analysis and interpretation of data. All authors made contributions to drafting and revising the article critically for important intellectual content. All authors approved the final version of the manuscript to be published.

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Conflict of interest

All authors declare no conflict of interest with regard to this trial.

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