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ORIGINAL ARTICLE *Andrology*

A short versus a long time interval between semen collection and intrauterine insemination: a randomized controlled clinical trial

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STUDY QUESTION: Does a short interval (i.e. ≤90 min), compared to a long interval (i.e. ≥180 min), between semen collection and intrauterine insemination (IUI) increase the cumulative chance of an ongoing pregnancy after six IUI cycles?

SUMMARY ANSWER: A long interval between semen collection and IUI resulted in a borderline significant improvement in cumulative ongoing pregnancies and a statistically significant shorter time to pregnancy.

WHAT IS KNOWN ALREADY: Retrospective studies assessing the effect of the time interval between semen collection and IUI on pregnancy outcomes have shown inconclusive results. Some studies have indicated a beneficial effect of a short interval between semen collection and IUI on IUI outcomes, while others have not found any differences. To date, no prospective trials have been published on this subject.

STUDY DESIGN, SIZE, DURATION: The study was performed as a non-blinded, single-center RCT with 297 couples undergoing IUI treatment in a natural or stimulated cycle. The study was conducted between February 2012 and December 2018.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Couples with unexplained or mild male subfertility and an indication for IUI were randomly assigned for up to six IUI cycles into either the control group (long interval, i.e. 180 min or more between semen collection and insemination) or the study group (short interval, i.e. insemination as soon as possible after semen processing and within 90 min of semen collection). The study was carried out in an academic hospital-based IVF center in the Netherlands. The primary endpoint of the study was ongoing pregnancy rate per couple, defined as a viable intrauterine pregnancy at 10 weeks after insemination.

MAIN RESULTS AND THE ROLE OF CHANCE: In the short interval group, 142 couples were analyzed versus 138 couples in the long interval group. In the intention-to-treat (ITT) analysis, the cumulative ongoing pregnancy rate was significantly higher in the long interval group (71/138; 51.4%) compared to that in the short interval group (56/142; 39.4%; relative risks 0.77; 95% CI 0.59–0.99; $P = 0.044$). The time to pregnancy was significantly shorter in the long interval group (log-rank test, $P = 0.012$). A Cox regression analysis showed similar results (adjusted hazard ratio 1.528, 95% CI 1.074–2.174, $P = 0.019$).

LIMITATIONS, REASONS FOR CAUTION: Limitations of our study are the non-blinded design, the long inclusion and follow-up period of nearly seven years and the large number of protocol violations, especially because they predominantly occurred in the short interval group. The non-significant results in the per-protocol (PP) analyses and the weaknesses of the study should be taken into account in the assessment of the borderline significance of the results in the ITT analyses.

WIDER IMPLICATIONS OF THE FINDINGS: Because it is not necessary to perform the IUI immediately after semen processing, there can be more time available to choose the optimum work-flow and clinic occupancy. Clinics and laboratories should find their optimal timing of insemination, considering the time between human chorionic gonadotropin injection and insemination in relation to the sperm preparation techniques used as well as the storage time and conditions until insemination.

STUDY FUNDING/COMPETING INTEREST(S): There were no external funding and no competing interests to declare.

TRIAL REGISTRATION NUMBER: Dutch trial registry, trial registration number NTR3144.
TRIAL REGISTRATION DATE: 14 November 2011.
DATE OF FIRST PATIENT'S ENROLLMENT: 5 February 2012.
Key words: intrauterine insemination / semen processing / time interval / randomized controlled trial / pregnancy

Introduction

Intrauterine insemination (IUI) is a minimally invasive, low-risk and cost-effective treatment option for couples diagnosed with mild endometriosis, unexplained subfertility, cervical factor subfertility or mild male factor subfertility (Ombelet *et al.*, 2014; Bahadur *et al.*, 2020). There are several factors influencing success rates in IUI, which can be roughly divided into clinical factors and technical factors. Clinical factors involve patient and cycle preparation characteristics, while the technical stage includes the processes between semen collection and insemination (Lemmens *et al.*, 2017). This stage is included in guidelines such as the WHO laboratory manual (World Health Orginization, 2021), but not every aspect is covered (Punjabi *et al.*, 2021). One of the topics where the literature is scarce and results are contradictory is the study of time intervals from semen collection to processing, from processing to insemination and, overall, from semen collection to insemination (Lemmens *et al.*, 2017).

Higher pregnancy rates were reported in IUI cycles when time intervals from semen collection to sperm wash, from sperm wash to IUI or from semen collection to IUI were shorter (Yavas and Selub, 2004; Kuru Pekcan, 2018; Punjabi *et al.*, 2021). Thresholds of $\leq$90 min (Yavas and Selub, 2004; Kuru Pekcan, 2018) or $\leq$107 min (Punjabi *et al.*, 2021) for the interval between semen collection and insemination were found to enhance pregnancy rates for both natural and stimulated IUI cycles. Furthermore, in a prospective cohort study, Fauque *et al.* (2014) found that an insemination time between 40 and 80 min post-semen processing resulted in the best pregnancy results.

In contrast to the aforementioned studies, Jansen *et al.* (2017) found no negative effect on ongoing pregnancy rates when insemination was delayed until the next day after semen processing. The group of Song *et al.* (2007) did not find any effect of the duration of the interval between semen collection and IUI on pregnancy results either.

To date, no randomized controlled trial (RCT) has been published on the subject of time intervals between semen collection and IUI. Due to the contradictory results and lack of randomized controlled studies in the literature, we conducted an RCT in which we investigated whether a short interval between semen collection and IUI increases the cumulative chance of an ongoing pregnancy after six IUI cycles, compared to a long interval.

Materials and methods

Study overview

The study was performed as a non-blinded, single-center RCT with patients undergoing IUI treatment in a natural or stimulated cycle. The study was conducted between February 2012 and December 2018 at an academic hospital-based IVF center in the Netherlands. The study protocol was approved by the Institutional Review Board of the VU University Medical Center, Amsterdam, the Netherlands (METc VUmc 2011/219). The trial was registered in the Dutch National Trial Registry (trial registration number NTR3144).

Participants

Couples with unexplained or mild male subfertility and an indication for IUI received verbal and written information about the study. Written informed consent was obtained if a couple was willing to participate. Couples were only allowed to participate before starting their first IUI cycle (or first IUI cycle after an ongoing pregnancy) within a treatment series of up to six IUI cycles. Couples who needed insemination with donor sperm or semen retrieved after a bladder flushing and women with polycystic ovary syndrome were not eligible. All couples underwent a fertility work-up, including an analysis of the menstrual cycle, a semen analysis and tubal testing (at least a hysterosalpingography). Mild male factor infertility meant that the total motile sperm count (TMC) after processing was $\geq$2 million and $<$10 million spermatozoa during fertility work-up.

Cycle/stimulation

The series of up to six IUI cycles started with three natural cycles followed by three stimulated cycles. However, during the study, the standard care for patients with unexplained subfertility changed from three natural and three stimulated cycles to six stimulated cycles due to a change in national guidelines. In stimulated cycles, ovarian stimulation was predominantly achieved with human menopausal gonadotropin (Menopur®; Ferring, Denmark) or, in fewer cases, with recombinant FSH (Gonal-f®; Merck Serono, Germany). In all IUI cycles, follicle growth was monitored by vaginal ultrasonography and serum estradiol determinations. Human chorionic gonadotropin (hCG) was administered when the diameter of the dominant follicle reached the size of $\geq$18 mm and the endometrial thickness was at least 6 mm. Ovulation tests were used directly after follicle size evaluation in the morning and/or prior to hCG injection in the evening, when a follicle was $\geq$18.5 mm. When the ovulation test was positive, the hCG injection was not administered. Cancelation criteria for insemination were $>$3 follicles of 18 mm, or $\geq$5 follicles of 14 mm, or an estradiol serum concentration of $>$3000 pmol/l. Insemination was performed at $\pm$42 h after hCG injection or $\pm$28 h after a positive ovulation test.

Semen preparation

Fresh semen was produced by the partner by masturbation predominantly (94%) in the clinic. Verbal and written instructions about the collection and transport (in case of production at home) of the semen sample were given in advance. Ejaculatory abstinence prior to the day of IUI of two to five days was advised. Semen processing was carried out at room temperature and started as soon as possible after liquefaction at 37°C. The first step was a centrifugation step using a single

layer medium: 70% PureSperm® (Nidacon, Sweden) and 30% human tubal fluid [HTF] hepes (Gynotec, the Netherlands) with 4 mg/ml human serum albumin [HSA] (Albuman, Sanquin, the Netherlands). Subsequently, a washing step was performed in HTF (Gynotec, the Netherlands) with 4 mg/ml human serum albumin [HSA] (Albuman, Sanquin, the Netherlands). For couples randomized in the long interval group, the pellet was then resuspended in HTF with 4 mg/ml HSA and stored at room temperature in an ultra violet light repellent box in 5% CO_2. Prior to insemination, one last centrifugation step was performed to concentrate the sample to a volume of 0.25 ml. For couples randomized in the short interval group, the last centrifugation step was carried out immediately after the washing step. Insemination was performed as soon as possible after the last centrifugation step. Before and after processing, a Makler Chamber was used to assess the sperm concentration (million/ml) and the percentage of motile sperm. Laboratory protocols and the type of media and disposables that were used did not change during the running of the study.

Endpoints and randomization

The primary endpoint of the study was ongoing pregnancy rate per couple, defined as a viable intrauterine pregnancy at 10 weeks after insemination. The secondary endpoints were (ongoing) pregnancy rate per cycle and percentage multiple pregnancies. The pregnancy outcomes of IUIs and natural conceptions were followed for six IUI cycles or for 12 months after the last IUI when less than six IUIs were performed.

Randomization (1:1) was performed by an independent researcher using computer generated random table numbers, with a block size of 20 and stratified for the indication of the IUI (mild male factor or unexplained subfertility). The allocations were placed in consecutively numbered, opaque envelopes. On the day of hCG injection of the first IUI cycle, patients were randomly assigned for all six IUI cycles into either the control group (long interval, i.e. 180 min or more between semen collection and insemination) or the study group (short interval, i.e. insemination as soon as possible after semen processing and within 90 min after semen collection). Due to the nature of the intervention, couples and caregivers were not blinded to group assignment.

Sample size

A long interval (≥180 min) between semen collection and insemination is our standard procedure. The ongoing pregnancy rate within six IUI cycles in our center is 39.4%. Yavas and Selub (2004) expected an increase of at least 13% in ongoing pregnancy rate per cycle, when insemination was carried out within 90 min after semen collection. This means a potential of a 56.6% cumulative rate of ongoing pregnancies after six IUI cycles. To detect this increase with a power of 80% at the two-sided 5% level of significance, 115 couples per arm were needed in each group. A withdrawal percentage of 10% of couples was anticipated, leading to an inclusion of at least 127 couples per arm.

Statistical analysis

Baseline characteristics of participating couples are described by frequency as percentages for categorical variables, by mean and SD for normally distributed continuous variables, and by median and interquartile range for non-normally distributed continuous variables. Differences in primary and secondary endpoints between the groups were tested with the Pearson chi-square test or the Fisher's exact test (in case of rare events). Relative risks (RR) with corresponding 95% CI are reported. Time to ongoing pregnancy was visualized by Kaplan–Meier curves and was compared between groups using discrete time-to-event analyses. Ongoing naturally conceived pregnancies after an IUI cycle were counted as ongoing pregnancy in the cycle after their last IUI cycle. For example, a pregnancy naturally conceived after three IUI cycles was counted as successful in the fourth cycle. Because no IUI was performed for these patients who naturally conceived, they were not added to the IUI cycle numbers in the fourth cycle. Couples who discontinued IUI were censored after their last IUI cycle. The main analyses were based on the intention-to-treat (ITT) principle; per-protocol (PP) analyses were performed as a sensitivity analysis. In the ITT analyses, all randomized participants were studied regardless of whether they completed the study or received another intervention instead of the assigned treatment. In the PP analyses, only data from those who strictly adhered to the study protocol were analyzed, i.e. all couples who discontinued planned treatment of six IUI cycles without reaching pregnancy were excluded. The PP analyses were performed to provide an estimate of the true efficacy of the intervention (short versus long interval).

Couples who were lost to follow-up were excluded, but best and worst case scenarios were imputed in a sensitivity analysis. Cox regression analyses for time to pregnancy as time axis and short or long interval group as event were performed with a correction for female age, female indication of subfertility, and female duration of subfertility.

All analyses were conducted in SPSS version 28 (IBM Corp., Armonk, NY, USA). Two-sided *P*-values <0.05 were considered as statistically significant.

Results

Between February 2012 and November 2017, a total of 467 couples were asked to participate in the study and 297 couples consented to participate (Fig. 1). These 297 couples were randomly assigned into either the short interval group (n = 149) or the long interval group (n = 148). Six women in the short interval group and nine women in the long interval group were randomized, although they did fulfill one of the exclusion criteria. These women were excluded from all analyses. Two women, one in each study arm, were lost to follow-up. A sensitivity analysis showed no differences in outcomes; therefore, these two women were excluded from all analyses as well. In total, 142 women were analyzed in the short interval group and 138 women were analyzed in the long interval group (ITT analysis). Follow-up ended in December 2018.

The reasons for not completing six cycles were predominantly due to naturally conceived pregnancies, advanced start of IVF/ICSI, protocol violations and personal reasons. Protocol violations were more frequent in the short interval group. During the study, 8 naturally conceived ongoing pregnancies occurred in the short interval group, compared to 13 in the long interval group.

The total number of IUI cycles in the short interval group was 598 (207 (34.6%) natural cycles) versus 495 IUI cycles in the long interval group (194 (39.2%) natural cycles).

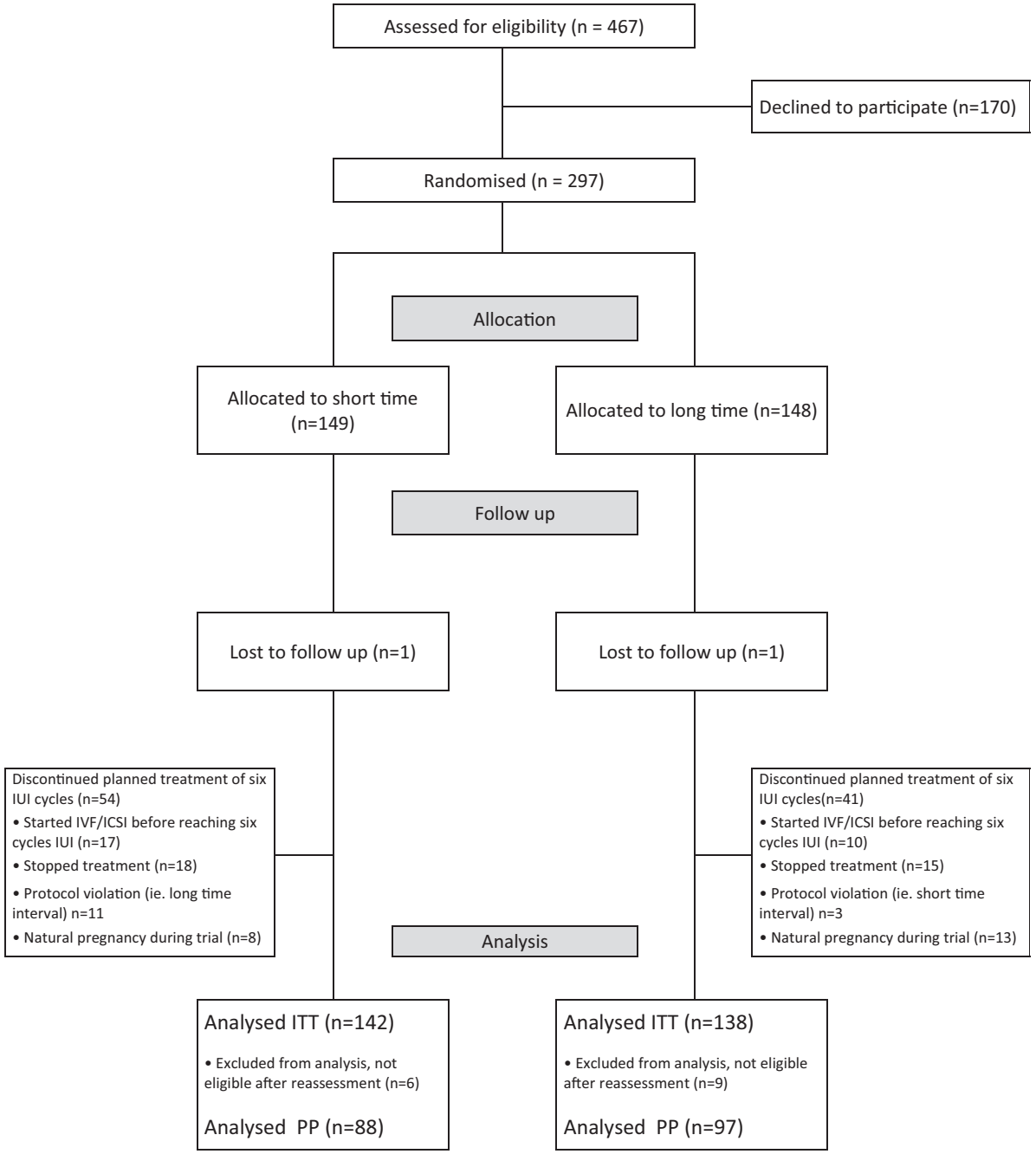


Figure 1. Participant flowchart.

Baseline characteristics were similar in the two study groups (Table I). Pregnancy outcomes are presented in Table II. Cumulatively, ongoing pregnancy was achieved in 56 couples in the short interval group and in 71 couples in the long interval group. In the ITT analysis, the cumulative ongoing pregnancy rate was significantly higher in the long interval group (71/138; 51.4%) compared to that in the short interval group (56/142; 39.4%); RR 0.77; 95% CI 0.59–0.99; $P = 0.044$. In the PP analysis, the cumulative ongoing pregnancy rate was not significantly different between the two study groups (RR 0.91; 95% CI 0.70–1.20; $P = 0.46$). The 95% CI for the difference in cumulative ongoing pregnancy rates (estimated proportions 39.4% and 51.4%) was −23.6% to −0.4%. In the ITT analysis, the ongoing pregnancy rates per cycle were 14.3% (71/495) in the long interval group and 9.4% (56/598) in the short interval group. This was significantly different (RR 0.65 [0.47–0.91]; $P = 0.011$). In the PP analysis, the ongoing pregnancy rates per cycle were alike

Table I Characteristics of study subjects included in the study.

Descriptive data	Short time interval	Long time interval
Number of participants	142	138
Female age at first IUI (years)	34.9 (3.9)	34.5 (4.2)
Maternal BMI (kg/m^2)	22.5 [20.8–24.2]	21.8 [20.4–25.1]
Unknown	3	1
Maternal smoking		
Smoker	18 (12.7)	23 (16.7)
Non-smoker	124 (87.3)	115 (83.3)
Maternal drinking		
Drinker	103 (72.5)	87 (63.0)
Non-drinker	39 (27.5)	51 (37.0)
Maternal subfertility		
Primary	78 (54.9)	83 (60.1)
Secondary	64 (45.1)	55 (39.9)
Duration of subfertility (years)	2.3 (1.2)	2.4 (1.2)
Infertility based on		
Unexplained	124 (87.3)	121 (87.7)
Mild male factor	18 (12.7)	17 (12.3)
Male age at first IUI (years)	37.3 (5.8)	37.3 (6.1)
Paternal BMI (kg/m^2)	24.8 [23.4–27.1]	24.8 [23.1–26.6]
Unknown	0	1
Paternal smoking		
Smoker	23 (16.2)	29 (21.0)
Non-smoker	119 (83.8)	109 (79.0)
Paternal drinking		
Drinker	97 (68.3)	90 (65.2)
Non-drinker	45 (31.7)	48 (34.8)
Number of IUIs per couple	5 [3-6]	3 [2-6]
Characteristics IUIs		
Number of IUIs	598	495
Natural cycles	207 (34.6)	194 (39.2)
Ovarian stimulation cycles	391 (65.4)	301 (60.8)
Number of inseminated progressive motile spermatozoa	16.0 [6.7–36.0]	22.0 [8.0–48.0]
Follicle growth		
1 follicle ≥14 mm	408 (68.2)	346 (69.9)
>1 follicle ≥14 mm	189 (31.6)	148 (29.9)
Unknown	1 (0.2)	1 (0.2)
Endometrial thickness	9.0 (1.8)	8.7 (1.8)
Unknown	3	2
Time interval semen production to IUI (min)	75 [67–84]	200 [184–214]

Values are n with percentage, mean with standard deviation or median values with interquartile range.
IUI: intrauterine insemination.

between the study groups (RR 0.82 [0.57–1.2]; $P = 0.27$). The multiple gestations per ongoing pregnancy were similar in both groups (RR 0.65 [0.19–2.2]; $P = 0.54$).

In the first three cycles, there were no differences in ongoing pregnancy rates between women with a natural cycle (31/212; 25.6%) and women with a stimulated cycle (41/134; 30.6%); OR = 1.279; 95% CI 0.714–2.307, $P = 0.4055$. Time to ongoing pregnancy is shown in the Kaplan–Meier curve in Fig. 2. Couples in the long interval group had a significantly shorter time to ongoing pregnancy: the discrete time survival analysis showed a significant difference in favor of the long interval group (log-rank test, $P = 0.012$). A Cox regression analysis, which was performed after visually checking the proportional hazard assumption,

Table II Outcome of intrauterine insemination.

	Short time interval n (%)	Long time interval n (%)	All n (%)	Relative risk (RR)	*P*-Value
Outcome per couple					
Number of participants: ITT	142	138	280		
Ongoing pregnancy	56 (39.4)	71 (51.4)	127 (45.4)	0.77 (0.59–0.99)	0.044[a]
Multiple gestation/ongoing pregnancy	4 (7.1)	6 (8.5)	10 (7.9)	0.65 (0.19–2.2)	0.54[b]
Couples with at least one miscarriage	10 (7.0)	7 (5.1)	17 (6.1)	1.4 (0.54–3.5)	0.49[a]
Number of participants: PP	88	97	185		
Ongoing pregnancy	47 (53.4)	57 (58.8)	104 (56.2)	0.91 (0.70–1.2)	0.46[a]
Multiple gestation/ongoing pregnancy	4 (8.5)	5 (8.8)	9 (8.7)	0.88 (0.25–3.1)	1[b]
Couples with at least one miscarriage	4 (4.5)	6 (6.2)	10 (5.4)	0.74 (0.21–2.5)	0.75[b]
Outcome per cycle					
Number of cycles ITT	598	495	1093		
Ongoing pregnancy	56 (9.4)	71 (14.3)	127 (11.6)	0.65 (0.47–0.91)	0.011[a]
Number of cycles PP	383	379	762		
Ongoing pregnancy	47 (12.3)	57 (15.0)	104 (13.6)	0.82 (0.57–1.2)	0.27[a]

[a]tested with chi-square test.
[b]tested with Fisher's exact test.
ITT: intention-to-treat analysis; PP: per protocol.

while adjusting for female age, female indication of subfertility and female duration of subfertility, led to similar conclusions as the Kaplan–Meier analyses. The adjusted hazard ratio for the long interval group (reference group: short interval group) was 1.528, 95% CI 1.074–2.174, $P = 0.019$.

Discussion

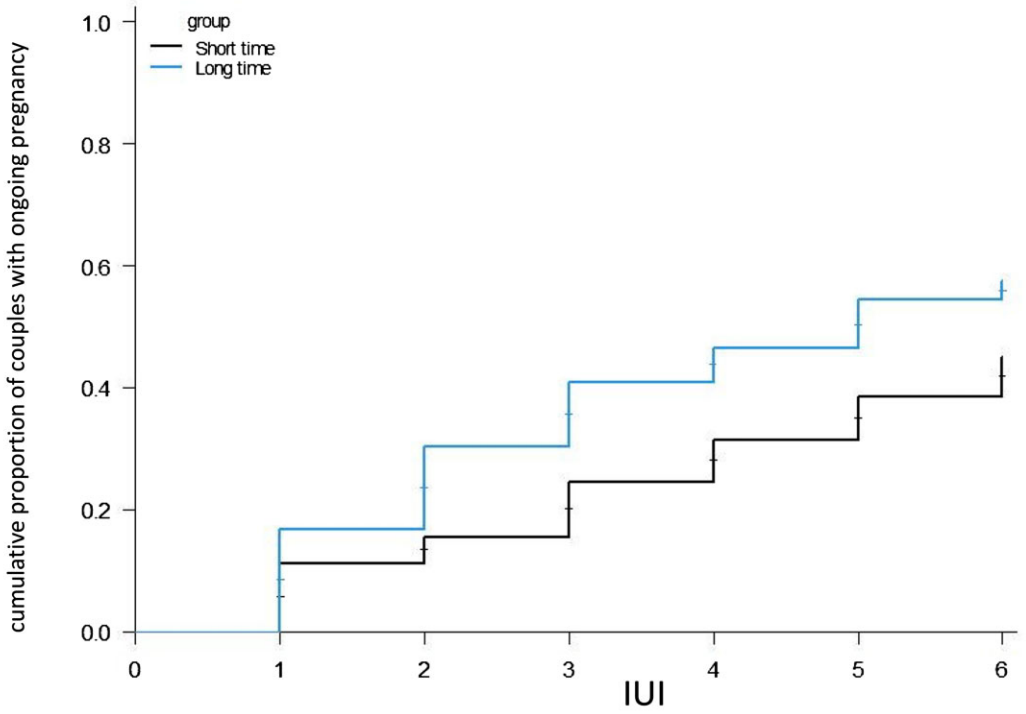
This RCT, evaluating a long versus a short interval between semen collection and insemination, showed a borderline significant improvement in cumulative ongoing pregnancies and a shorter time to pregnancy in the long interval group. Multiple pregnancies and miscarriages were comparable between the two groups.

The results of our RCT are different from results of previous published, retrospective studies. These retrospective studies either found no effect of the duration of the interval between semen collection and insemination (Song *et al.*, 2007; Jansen *et al.*, 2017) or a beneficial effect of a shorter time between semen collection and IUI (Yavas and Selub, 2004; Fauque *et al.*, 2014; Kuru Pekcan, 2018; Punjabi *et al.*, 2021).

A possible mechanism of the effect that a longer interval between semen collection and insemination is beneficial might lie in a combination of factors regarding the ability of the oocyte to become fertilized and ability of the spermatozoa to fertilize an oocyte. Correct timing of the insemination is vital, as oocytes and spermatozoa have a limited survival time (Cantineau *et al.*, 2014). Therefore, to establish the correct timing of insemination, the conditions concerning both the oocyte as the spermatozoa should be considered.

Concerning the oocyte, the ovulation time and its interval to IUI have been studied previously. Although there is still no consensus on the optimal time interval between hCG injection or LH surge and insemination, this interval can be influential on IUI outcomes (Firouz *et al.*, 2020). Several RCTs studying a long versus a short interval between hCG injection and insemination have been published. Analyzing different short (insemination at the point of hCG and 24 h after hCG) and long (insemination 36 h after hCG and 48 h after hCG) intervals, some of these studies have found comparable pregnancy results between the long and short groups (Rahman *et al.*, 2011; Rijsdijk *et al.*, 2019), while others have found better pregnancy outcomes in the long group (Firouz *et al.*, 2020). However, little information is presented about the work-up methods of the semen and/or the time between semen processing and insemination in these studies. Intercourse within days of the insemination was either not recorded or even recommended, introducing bias to the results.

Concerning the spermatozoa, it is known that spermatozoa first need to mature functionally in the process of capacitation before they can fertilize an oocyte. Capacitation involves a complex sequence of events in which the external environment plays a crucial role (Fraser, 1998). Temperature and type of incubation medium have an important role in the time and degree of capacitation. It is known that long-term storage ($\geq$24 h) of spermatozoa at room temperature results in a better preservation of sperm quality than long-term storage at 35°C. This might be due to spermatozoa going into a rest state which allows them to preserve their energy (Thijssen *et al.*, 2014). Incubation of spermatozoa at room temperature does not allow capacitation, but this temporary blockage disappears when spermatozoa are exposed to a 37°C temperature (Marin-Briggiler *et al.*, 2002). Moseley (2005) showed that 90 min incubation of spermatozoa in IVF medium (a bicarbonate buffered medium) at 37°C accelerated sperm capacitation compared with a standard capacitation medium. In addition, an IVF sperm buffer (HEPES-buffered solution containing bicarbonate, a medium similar



cycle number	1	2	3	4	5	6
long	20/136	20$^{\$}$/111	8*/85	9$^{\$}$/63	11$^{\$}$/55	3/45
short	15/141	6*/122	11*/114	10$^{\#}$/88	4/76	10^/57

Figure 2. Discrete time–event survival curve expressing time to ongoing pregnancy. The black line represents the short interval group. The blue line represents the long interval group. Below the Kaplan–Meier curves, the numbers of ongoing pregnancies per number of IUI cycles are presented. *Including one naturally conceived ongoing pregnancy. $^{\#}$Including two naturally conceived ongoing pregnancies. $^{\$}$Including three naturally conceived ongoing pregnancies. ^ Including four naturally conceived ongoing pregnancies. $^{\&}$Including six naturally conceived ongoing pregnancies.

to most commercial sperm wash media), did not stimulate sperm capacitation in those incubation conditions.

In our study, prior to IUI, spermatozoa were stored for a short or a long time in bicarbonate buffered medium (similar to IVF medium) at room temperature. Although room temperature (temporarily) does not allow capacitation, the medium used does enhance capacitation at 37°C. We hypothesize that the storage medium might have started the process of capacitation at ambient temperature in the long group, allowing the spermatozoa to complete capacitation within a short period of time after insemination. The spermatozoa in the short group might need more time to complete capacitation and therefore, they might need more hours to be competent to fertilize an oocyte. The insemination in our study was performed at 42 h after hCG injection, i.e. shortly before or directly after time of ovulation. This combination of factors might have resulted in a lower degree of fertilization in the short group and hence fewer pregnancies because in the short group, fewer spermatozoa were capacitated at the time of ovulation and therefore most were not able to fertilize the oocyte yet.

Alternatively, the reason that the long interval group resulted in significantly more ongoing pregnancies per couple and a shorter time to pregnancy might be due to chance. The significance of the ongoing pregnancy rates is borderline, the beneficial effect is not seen in the PP analysis and, in addition, in the long interval group, there were more naturally conceived pregnancies which might have been the cause of the significant difference in the ITT analysis. A further indication of the role of chance are the results of the ongoing pregnancy rates in the long interval group (51.4%) that outperform our center's historic ongoing pregnancy rate (39.4%), which was used for the power calculation.

Limitations of our study are the long inclusion and follow-up period of nearly seven years and the large number of protocol violations. During the length of the study, we did alter one item in the IUI protocols for the study patients. The standard care for patients with unexplained subfertility changed from three natural and three stimulated cycles to six stimulated cycles. However, this alteration was introduced at one specific time point (not gradually) and the participants under each protocol were equally distributed between the two study groups. The absence of a statistically significant difference in ongoing pregnancy rates in the first three IUI cycles between women with a natural cycle and women with a stimulated cycle is a further indication that it is highly unlikely that the switch to six stimulated cycles has been of influence in

the data. The overall pregnancy rate per cycle of our center, the clinical work-up methods, laboratory protocols and semen preparation media and disposables did not alter during the length of the study. The study design did cause a certain amount of discontinuation of the planned treatment because of naturally conceived pregnancies, social reasons and treatment alterations. Nevertheless, the study design does mirror daily clinical practice where a certain number of 'drop-outs' is expected. The large number of protocol violations is a major limitation of our study, especially because they predominantly occurred in only one of the study arms, i.e. the short interval group. Despite all precautions taken and training of staff, because of the many cycles involved and because of a change of ICT system, numerous of cycles in the short interval group were overlooked at the time of IUI planning and the standard way of IUI (i.e. a long interval) was assigned to some patients and cycles in the short interval group. Because this misscheduling occurred randomly, it is highly unlikely that this caused systematic differences in patient characteristics of the patients with these protocol violations. The study design also lead to a heterogeneity in the patient population. Although this mimics daily patient care and the different factors did not differ significantly between the study groups, it also makes it more difficult to draw conclusions for specific patient populations. More research is necessary to study the effect of the time interval between semen production and IUI in more detailed patient groups.

Another limitation of our study is the non-blinded design, but blinding is impossible for the participant and the laboratory technician for the interval between semen collection and insemination. And because the IUI in the short interval group was performed at a different time compared with the long interval group (conveniently chosen for the men who could consequently produce semen before going to work), the doctor was not blinded either.

The results of our study may have implications for daily practice of the planning and timing of IUIs. Because we have shown that it is not necessary to perform the IUI immediately after semen processing, there is more time available to choose the optimum work-flow and clinic occupancy.

In conclusion, in our clinic and laboratory settings, a long interval ($\geq$180 min) between semen collection and insemination, resulted in a borderline significantly higher cumulative ongoing pregnancy rate per couple and a shorter time to pregnancy. The PP analysis did not show a significant difference in cumulative ongoing pregnancy rates between the short and the long group, which might give an indication of the direction (i.e. not significant) of the borderline results in the ITT analyses. The timing of IUI must include a combination of factors concerning the ability of the oocyte to get fertilized and ability of the spermatozoa to fertilize an oocyte. We therefore advocate that every clinic and laboratory should find their optimal time of insemination, considering the time between hCG injection and insemination in relation to the sperm preparation techniques used as well as the storage time and conditions until insemination.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

C.H.S.-L.: study conception and design, acquisition of data, analysis and interpretation of data, and manuscript reviewing. R.S.: study conception and design and acquisition of data. B.I.L.W.: statistical analysis, analysis and interpretation of data and writing. E.H.K.: interpretation of data, and manuscript reviewing. C.B.L.: study conception and design, analysis and interpretation of data, and manuscript reviewing. C.G.V.: study conception and design, analysis and interpretation of data, and manuscript writing and reviewing.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding authorship or publication of this study.

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