Intrauterine insemination: is the timing correct?

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Abstract

Objective To evaluate the impact of follicle rupture on pregnancy rate in intrauterine insemination.

Material and method Retrospective cohort study. Evidence of follicle rupture was assessed by transvaginal ultrasonography following IUI. Two hundred ninety-six couples with unexplained infertility and 121 couples with male factor subfertility were included. Results of 578 cycles were analyzed here.

Result Clinical pregnancy rate was 23.5% (64/272) in the group when follicle rupture was evident by transvaginal ultrasonography, while it was only 8.8% (27/306) when follicle rupture was not evident ($p < 0.001$).

Conclusion Postponing IUI until observation of follicle rupture may yield a higher pregnancy rate.

Keywords Intrauterine insemination · Timing · Follicle rupture · Pregnancy rate

Introduction

Timing of intrauterine insemination (IUI) is a controversial issue in couples undergoing infertility treatment. Despite all efforts aimed at improving pregnancy rates, overall success of IUI is still not satisfactory. Although pregnancy rates vary widely among programs, it is usually accepted that a 10% success is reasonable for an IUI treatment combined with controlled ovarian stimulation (COS) [1].

Prediction of an ongoing pregnancy after intrauterine insemination was studied with a multicenter study [2]. Including 14,968 cycles of 3,371 couples, it was concluded that increasing maternal age, longer duration of subfertility, presence of male factor subfertility, one-sided tubal pathology, endometriosis, uterine anomalies and an increasing number of cycles were unfavorable predictors for an ongoing pregnancy. Cervical factor and the use of ovarian hyperstimulation were shown to be favorable predictors. On the other hand, Ahinko-Hakamaa et al. have found other factors such as follicle number [3]. They reported that in combined infertility the highest pregnancy rate was with sequential clomiphene citrate/human menopausal gonadotrophin stimulation and with three follicles.

The occurrence of ovulation as indicated by follicle rupture was never studied before. The aim of this study was to evaluate whether timing of IUI in relation to the rupture of the dominant follicle affected the probability of pregnancy following COS + IUI.

Material and method

This study was performed in a private center of assisted reproduction. Institutional review board approval was obtained. Data of 641 IUI cycles in 417 couples between the years 2000 and 2006 were analyzed retrospectively. Etiology of infertility was unexplained in 296 couples and of male origin in 12. Couples with other causes of infertility such as documented endometriosis were excluded because of possible detrimental effects on ovulation, fertilization, and pregnancy. Of 417 couples, 301 had three cycles of ovulation induction with clomiphene citrate previously. Seventy-four women had undergone diagnostic laparoscopy for infertility. The mean age of female partner was 28.4 ± 6.9 years (range

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22–35). Controlled ovarian stimulation was undertaken with follicle stimulating hormone injections tailored individually for each woman. Criteria for human chorionic gonadotropin (hCG) injection was the diameter of leading follicle exceeding 17 mm. Ejaculate was obtained by masturbation following 3 to 7 days of abstinence. Semen was prepared with a two-layer density gradient (45% and 90%) separation technique (Puresperm; Nydacon, Gothenburg, Sweden), and the final sample was suspended in 0.5 mL of culture media for insemination. Intrauterine insemination was performed 36–38 h after the injection of 10,000 IU hCG. Insemination was performed with a commercial catheter (IUI, CCD Laboratories, Paris, France). Cervical mucus was not aspirated before insemination. Intrauterine inseminations were performed at dorsal lithotomy position. Following IUI, transvaginal ultrasonography was performed to check whether the dominant follicle had ruptured, as indicated by follicular collapse and fluid accumulation in the pouch of Douglas. The patients were advised to rest for 30 min prior to being discharged. No luteal support was given. In order to test the effectiveness of IUI treatment, the couples were advised to refrain from sexual intercourse until the next visit 2 weeks later.

Results

Of 641 cycles, 578 were included in the final analysis. Reasons for exclusion were low progressive motile spermatozoa count ($<5 \times 10^6$) after semen preparation ($n=48$), failure to insert the IUI catheter ($n=7$), failure to produce a semen sample [2], failure to timely injection of hCG ($n=4$) and switching to rescue IVF ($n=2$). Figure 1 displays the flow diagram of the study.

Follicular rupture was detected by transvaginal ultrasonography at the time of IUI in 272 cycles, whereas no sign of rupture was observed in 306 cycles. There were 64 pregnancies in rupture detected group (23.5%) and 27 pregnancies in the no-rupture group (8.8%) ($p<0.001$). Table 1 displays comparisons between the subgroups according to follicle rupture and to the clinical outcome. There was no statistically significant difference between the subgroups in female age, day 3 FSH level, duration of infertility, total motile sperm count and number of follicles >17 mm.

Discussion

Current study demonstrated that clinical pregnancy rate was significantly higher when follicle rupture was detected by transvaginal ultrasonography compared to that of when follicle rupture was not evident. Occurrence of follicle

Fig. 1 Flow-chart of the study

rupture provided 23.5% pregnancy rate, which was similar to natural fecundity rate.

In stimulated cycles, ovulation usually occurs 36 h after hCG administration [4]. Based on this finding, IUI has been traditionally performed around post-hCG 36th hour. Guzik et al. have reported 33% pregnancy rate when IUI was performed between 36–40 h after hCG injection [5]. Two hCG-IUI intervals, 33 versus 39 h, was compared in a prospective, randomised clinical trial [6]. There was no statistically significant difference in pregnancy rates (21% versus 15%) between short and long hCG-IUI interval groups.

An alternative approach to achieve the correct timing is to increase the number of inseminations per cycle from one to two. The rationale behind this strategy is to provide more motile spermatozoa to be present at the ampulla of the Fallopian tubes during the fertilizable period of the oocyte. However, studies have yielded controversial results. Ragni et al. analyzed 449 cycles of 273 couples undergoing COS with clomiphene citrate combined with IUI [7]. Double insemination performed at 12 and 34 h after hCG provided significantly higher pregnancy rate compared to single IUI or double IUI at 34 and 60th hour after hCG administration. On the contrary, in a Cochrane review which included two trials, Cantineau et al. reported no significant benefit of double IUI over single IUI, based on the results of pregnancy rate per couple [8]. A broader review by Osuna et al. also showed that double insemination was not superior to single insemination [9].

It is thought that spermatozoa survive for 40–80 h in the female reproductive tract [10]. However, this holds true for

Table 1 Comparisons of subgroups

	Follicle rupture cycles <i>n</i> :272			No rupture cycles <i>n</i> :306			<i>p</i> Sub A vs Sub C	<i>p</i> Sub B vs Sub D	<i>p</i> Sub A + Sub B vs Sub C + Sub D
	Pregnant Sub A <i>n</i> :64	Non-pregnant Sub B <i>n</i> :208	<i>p</i>	Pregnant Sub C <i>n</i> :27	Non-pregnant Sub D <i>n</i> :279	<i>p</i>			
Age (years)	27.7±5.5	28.7±4.9	NS	28.2±6.9	26.7±4.2	NS	NS	NS	NS
Day 3 FSH (IU/L)	7.2±1.9	7.4±2.2	NS	6.9±2.1	8.2±1.1	NS	NS	NS	NS
Duration of infertility (months)	21.5±4.0	24.2±2.9	NS	22.5±5.2	23.8±3.2	NS	NS	NS	NS
TMS (10 ⁶)	19.2±6.6	22.1±5.4	NS	21.2±8.2	26.1±7.8	NS	NS	NS	NS
No of follicle >17 mm	2.4±1.1	2.4±1.7	NS	2.9±1.2	2.1±1.2	NS	NS	NS	NS
Pregnancy	64/272			27/306			<0.001		

Comparisons were made by using χ^2 test and student *t* test where appropriate
Sub A Subgroup A, *Sub B* Subgroup B, *Sub C* Subgroup C, *Sub D* Subgroup D

spermatozoa residing in cervical mucus. On the other hand, following IUI, inseminated spermatozoa do not stay in reproductive tract for such a long time. In less than an hour, inseminated spermatozoa will be spilling out of tubal ostia into the peritoneal cavity, where inflammatory reaction occurs and strong phagocytic activity is present.

Spermatozoa have been shown by laparoscopy to be present in the pouch of Douglas 2–4 h after IUI [11, 12]. Considering the sperm velocity of 1 cm per min, spermatozoa might have reached peritoneal cavity even earlier. Theoretically, the inseminated aliquot of sperm should be spilling out of tubal ostia 15–20 min after insemination.

The recommended minimum number of progressive motile spermatozoa to be present for insemination after semen preparation varies between 0.8 to 10×10^6 in previous reports [13]. The cycles in which less than 5×10^6 motile spermatozoa present after semen preparation, were excluded from this study. As low motile sperm count is among confounding factors in the success of IUI treatment. This exclusion helped us to purify the study group to analyze only the impact of follicle rupture on pregnancy rate.

The incidence of luteinized unruptured follicle syndrome (LUF) has been reported to be 10% among fertile women [14]. It is reported to be as high as 25% in unexplained infertile couples undergoing IUI with clomiphene citrate [15]. LUF decreases the chance of pregnancy after intrauterine insemination treatment. Ultrasound has been demonstrated to be the method of choice for diagnosis of LUF [16]. It is not possible to comment on the incidence of LUF in this series since the monitoring of the patients was not extended beyond 38th hour after insemination. Postponement of IUI until demonstration of follicular rupture will avoid insemination in the presence of LUF and such a strategy may increase the pregnancy rate per inseminated cycle with IUI treatment.

In a prospective study, 32 patients were monitored by serial ultrasonographic examinations with 1 h intervals

after hCG administration [17]. Immediate IUI upon observation of follicular rupture resulted in a pregnancy rate of 16%. Leading follicle was not the first to rupture in 32% of patients. Insemination was not postponed until the largest follicle ruptured, which probably harbored the most fertilizable oocyte. It can be speculated that the pregnancy rates could have been higher had the authors waited for the dominant follicle to rupture. The small sample size was considered to be another drawback in this study.

According to World Health Organization analysis of natural cycles, ovulation takes place from 24 to 56 h after the onset of the LH surge, with a mean time of 32 h [18]. Timing of ovulation in stimulated cycles also varies considerably, and depends on the ovarian stimulation protocol used. Oocytes are fertilizable for only a period of 12–16 h [19]. It has been shown that the period when an oocyte can be successfully fertilized begins 6 h after ovulation [20]. This means that, even if spermatozoa meet the oocyte in the Fallopian tube at the time of ovulation, there still are 6 h to the fertilizable period of the oocyte to start. This suggests that IUI after observation of ovulation should be preferred to IUI prior to or even to the exact time of ovulation.

Since this is a retrospective study, a prestudy sample size calculation was not performed. Post-hoc power calculation was undertaken. Any intervention which might increase the success of IUI from 10% to natural fecundity rate of 20% was deemed worthy. The number of cases to be included in the study was calculated as 199 in each arm, with the use of the commonly accepted probability of type I and type II errors set at 0.05 and 0.20, respectively [21]. Considering these criteria, the current study has the sufficient power (=1) to draw reliable conclusions.

In conclusion, the results of the current study suggests that monitoring of follicular rupture prior to IUI provides a pregnancy rate similar to natural fecundity. According to

the results of this study it is suggested that IUI should be withheld until follicular rupture is detected.

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