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ORIGINAL ARTICLE *Infertility*

The effects of timing of intrauterine insemination in relation to ovulation and the number of inseminations on cycle pregnancy rate in common infertility etiologies

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BACKGROUND: Controlled ovarian hyperstimulation with intrauterine insemination (COH/IUI) is an established tool in medically assisted conception for many infertility factors. However, the proper timing of IUI after hCG trigger and the frequency of IUI are still debated. We aimed to examine the association between the cycle pregnancy rate (CPR) and: (i) single IUI timed at 36 $\pm$ 2 h post-hCG (pre- or post-ovulation) (ii) the number of IUI (single or double) for pre-ovulatory cases both aims in male, anovulatory and unexplained infertility.

METHODS: The study included a total 1146 first-stimulated cycles in infertile couples due to male factor, anovulation or unexplained infertility. Cycles were stimulated by clomiphine citrate (CC) or sequential CC–hMG or hMG and monitored by transvaginal ultrasound. When the leading follicle reached $\geq$18 mm mean diameter, 10 000 IU hCG was given to trigger ovulation and IUI was timed for 36 $\pm$ 2 h later. Semen was processed and ovulation was checked at the time of IUI. Post-ovulatory cases received single IUI, while pre-ovulatory cases were sequentially randomized to receive either single or double IUI. The end-point of the cycle was CPR.

RESULTS: Overall CPR in the whole cohort was 10.1%. When ovulation was present before IUI, CPR was 11.7% compared with 6.7% when ovulation was absent [OR (95% CI): 1.85 (1.12–3.06), $P = 0.015$]. When this OR was computed according to infertility etiology, it was 1.26 (0.52–2.95) ($P = 0.82$) for male factor infertility and 2.24 (1.23–4.08) ($P = 0.007$) for non-male factor infertility. Comparing the CPR for double versus single IUI in pre-ovulatory cases, the OR for all cycles was 1.9 (0.76–4.7) ($P = 0.22$), but according to etiology, it was 4.667 (0.9–24.13) ($P = 0.06$) in male factor and 1.2 (0.43–3.33) ($P = 0.779$) for non-male factors.

CONCLUSIONS: Single IUI timed post-ovulation gives a better CPR when compared with single pre-ovulation IUI for non-male infertility, whereas for male factors, pre-ovulation, double IUI gives a better CPR when compared with single IUI.

Key words: infertility / intrauterine insemination / ovarian stimulation

Introduction

Intrauterine insemination (IUI), with or without controlled ovarian hyperstimulation (COH), is an indispensable part of infertility treatment because it is a non-invasive and often successful procedure (Prietal *et al*., 2001). It is a cost-effective treatment for most subfertile couples and should therefore be offered as a first-line treatment option (Cohlen, 2005). Moreover, IUI in stimulated cycles may be considered while a waiting IVF or in women with patent tubes when IVF is not affordable (The ESHRE Capri Workshop Group, 2009). However, many aspects that could optimize the rate of success of IUI remain to be defined. Among debated issues are the correct timing of insemination (Ragni *et al*., 2004), the impact of follicle rupture at the time of IUI (Kucuk, 2008) and the number of

inseminations per cycle (Guzick, 2004). No difference has been detected among different techniques for timing IUI (detection of LH in urine or blood, hCG administration, combination of LH detection and hCG administration, basal body temperature chart, ultrasound detection of ovulation, GnRH agonist administration), as shown by a recent Cochrane review (Cantineau *et al*., 2010). The timing of IUI, in the majority of published studies, is 32–36 h following hCG administration (The ESHRE Capri Workshop Group, 2009). As regards the impact of the number of inseminations on cycle pregnancy rate (CPR), the majority of available studies have reported no significant differences between single and double IUI (Cantineau *et al*., 2003; Alborzi *et al*., 2003; Gezginç *et al*., 2008; Bagis *et al*., 2010), while a few studies (Duran *et al*., 2002; Osuna *et al*., 2004) have suggested better cycle outcome with double IUI. Although it is assumed that the timing of insemination relative to ovulation is critical for an optimal success rate, we found only one retrospective study of mainly unexplained infertility (Kucuk, 2008) which addresses this topic. The effects of the timing of IUI relative to ovulation in different infertility etiologies and the effect of the infertility diagnosis on cycle outcome using single and double IUI have been insufficiently studied. Our aims were to study: (i) the association between the timing of single IUI (pre- or post-ovulation) and the CPR in male, unexplained and anovulatory infertility etiologies, and (ii) the association between the number of inseminations (single or double) and the CPR in cycles with an unruptured follicle at 36 $\pm$ 2 h in the above infertility etiologies.

Materials and Methods

Patients

This prospective cohort study was carried out at a private infertility center during the period May 2007 to May 2010. The whole cohort included 1238 first IUI cycles for the following indications: male factor, anovulation, endometriosis, combined male and female factors and unexplained infertility. The endometriosis and combined male–female factor groups were excluded because of the heterogeneity of cases in both groups. In the endometriosis group, there were varied degrees of severity and in the combined male and female factor group, there were different female factors. Another reason for exclusion is the small number of cycles in the endometriosis and combined male–female factor groups, which would render statistical analysis of limited value (both factors combined represent 8% of the whole cohort). The study therefore included these groups: male factor (428 cycles), anovulation (226 cycles) and unexplained infertility (492 cycles). Semen samples were classified as fertile or subfertile in accordance with the WHO standards (WHO Laboratory Manual, 1999) for sperm concentration and motility, and with Kruger's strict criteria for sperm morphology. Two semen analyses were reviewed with an interval of at least 3 months. The prerequisites for inclusion in the subfertile male group in the study was the presence of motile sperm with counts $>1 \times 10^6$/ml in the fresh specimen and a normal sperm morphology of $\geq$5% with a normal female partner. Exclusion criteria for male factor were: ejaculatory dysfunction, semen parameters below the above-mentioned lower cut-off values and presence of infertility factors in the wife. A universal threshold level of semen parameters above which IUI can be performed with acceptable pregnancy rates has not been determined yet (Ombelet *et al*., 2003; The ESHRE Capri Workshop Group, 2009) and since many of the couples with severe male factor could not afford ICSI, the only available option for them was attempting IUI. Polycystic ovary syndrome (PCOS) was defined according to the modified Rotterdam revised ESHRE/ASRM criteria (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group revised, 2004). Only infertile PCOS women seeking pregnancy through ovulation induction with IUI were included. Exclusion criteria of PCOS infertile cases were the presence of subfertile semen parameters in the husband. The diagnosis of unexplained infertility was based on normal findings in seminal fluid analysis, mid-luteal serum progesterone and hysterosalpingogram or laparoscopy. As mentioned before endometriosis, combined male and female factor infertility, and combined female factors were excluded from the study. The study thus included a total of 1146 consecutive stimulated first cycles of COH/IUI using the husband's semen for infertile couples who were judged to be candidates for COH/IUI.

Procedures

The ovarian stimulation protocol consisted of clomiphine citrate (CC), sequential CC–hMG or exclusive hMG. The sequential CC–hMG protocol was the most frequently used type. Oral tablets of CC (50 mg) were given in doses of 100 mg/day from the third to seventh day of the cycle. In the sequential CC–hMG protocol, CC was followed by hMG sequentially from the eighth day. The hMG dose was titrated against ovarian response to obtain one to four follicles of 18–20 mm mean diameter as shown by serial transvaginal sonographic (TVS) monitoring started on the 10th day of the cycle and repeated every other day until the day of ovulation triggering. The ovulatory hCG dose (10 000 IU) was given when mean diameter of the leading follicle reached $\geq$18 mm. Cycles were cancelled when large follicles (mean diameter $\geq$16 mm) were more than four in number (to avoid multiple pregnancies) and/or when medium-sized follicles (mean diameter 12–15) were $\geq$10 in number (to avoid hyperstimulation syndrome). Owing to sequential use of CC and hMG, and the variable hMG dose regimen used, rarely hCG administration had to be cancelled. The insemination procedure was scheduled for 34–38 h after hCG injection (i.e. 36 $\pm$ 2 h) and the timing of the hCG dose was adjusted to be at 10 p.m. $\pm$2 h to allow insemination to be in the laboratory working hours (8 a.m. to 2 p.m.). At the time of insemination, the occurrence of ovulation was checked by TVS before the procedure. If ovulation was diagnosed (evidence of follicular rupture as shown by the presence of free fluid in Douglas pouch and visible corpus luteum and/or disappearance of follicles) insemination was done once. If ovulation had not occurred yet, patients were randomly assigned to either repeat insemination (double IUI) 24 h later (when the calendar date was even number) or not to repeat insemination (single IUI) (odd number calendar date). After insemination, the patient was given luteal support in the form of micronized progesterone 400 mg vaginal tables per day and a pregnancy test (serum β-hCG level) was scheduled 14 days later. Luteal support was given to all cases irrespective of the stimulation protocol. The endpoint of the treatment cycle was either a negative pregnancy test or a positive test confirmed by clinical evidence of pregnancy in the form of intrauterine gestational sac visualized by ultrasound 2 weeks after positive biochemical test. The study was accepted by the center's ethics committee and informed consent of couples was obtained before inclusion in the study.

Semen processing

The husband was instructed to abstain from sexual intercourse for 3 days before insemination. Semen was obtained by masturbation in a sterile plastic container. After liquefaction, semen was processed using the swim-up method (if semen was normal) or mini swim-up method (if semen was subfertile). In the swim-up method, 1 ml of insemination media (IVF media Ferti Cult/IVF/FIV, FertePro N.V, Belgium) is mixed with an equal volume (1:1) of semen, while in the mini swim-up

Table I Cycle outcome related to occurrence of ovulation at IUI 36 ± 2 h post–hCG.

Parameter	All cycles	Male	Non-male		
			Total	Anovulation	Unexplained
Cycle outcome known	1146	428	718	226	492
Ovulation status known	1114	422	692	212	480
Ovulation present (*n*)[a]	816	322	494	146	348
Ovulation absent (*n*)[a]	298	100	198	66	132
Ovulation rate (%)	73.2	76.3	71.3	68.3	72.5
All cycles with clinical pregnancy (*n*)[a]	116	30	86	34	52
Overall CPR[b] (%)	10.1	7.1	12.4	16.1	10.8
Pregnancies in positive ovulation cycles (*n*)[a]	96	24	72	28	44
CPR[b] in cycles with positive ovulation (%)	11.7	7.4	14.6	19.1	12.6
Pregnancies in negative ovulation cycles (*n*)[a]	20	6	14	6	8
CPR[b] with negative ovulation (%)	6.7	6	7	9.9	6
OR (95% CI) of pregnancy in positive ovulation versus negative ovulation cycles	1.85 (1.12–3.06)	1.26 (0.52–2.95)	2.2 (1.23–4.08)	2.1 (0.91–4.85)	2.08 (1.01–4.31)
P	0.015[c]	0.82[c]	0.007[c]	0.071[c]	0.047[c]

[a]Number.
[b]Cycle pregnancy rate.
[c]χ^2 test.

method, the ratio is 1:3. The suspension is then centrifuged for 10 min at 300*g*, or 3–5 min at 500*g*. The mixture is kept for about 45 min at 37°C. Then the upper top 0.6 ml is separated in a test tube and insemination is carried out using a soft catheter (Gynetics: Genetics Medical Products NV, Hammont–Achel, Belgium). When soft catheter could not be passed through the internal os, a rigid catheter was used.

Statistical analysis

Statistical analysis was done using two-tailed Student's *t*-test for comparison between group means for normally distributed quantitative data, and χ^2 test and odds ratio with 95% interval (OR, 95% CI) for comparing proportions. Microsoft Excel 2003, SPSS 10.0 (SPSS, Chicago, IL, USA) for Windows was used in the analysis. For sample size calculation, Epi Info 2000 ver 1.1 2001 program (CDC Atlanta, GA, USA) was used. The outcome measures were CPR when IUI was performed after ovulation compared with IUI performed before ovulation, and the CPR with single IUI compared with double IUI in the different etiologic groups, and all comparisons were expressed as OR (95% CI). The difference is considered significant when $P < 0.05$. The sample size for the whole cohort was estimated for the first objective to be 812 completed cycles, 580 in post-ovulation and 232 in pre-ovulatory arms, assuming a 2.5:1 ratio between post-ovulation and preovulation at IUI 36 h post-hCG (for $\alpha = 0.05$, $\beta = 0.80$).

Results

This prospective cohort study comprised a total of 1146 completed first COH/IUI cycles for the indications shown in Table I. Out of these 1146 completed first cycles, ovulation status was documented in 1114 cycles; the missing 32 were owing to incomplete recording. Ovulation was observed at the time of IUI (36 ± 2 h post-hCG) in 816 out of these 1114 cycles (73.2%). The incidence of ovulation at IUI was nearly equal in different infertility etiologies (Table I). The overall CPR in the study is 116 of 1146, i.e. 10.1%. Comparing cycles with evidence of ovulation (11.7%) and those without ovulation (6.7%) at IUI for all cycles, CPR was significantly higher when ovulation was present: OR (95% CI) 1.85 (1.12–3.06) ($P = 0.015$). However, when this comparison was made for ovulation and non-ovulation in the male (7.4 versus 6.0%) and non-male (14.6 versus 7.0%) indications, the difference in CPR was not significant in the male-factor group: OR (95% CI) 1.26 (0.52–2.95) ($P = 0.82$), but it was significant in favor of follicular rupture in the whole non-male indication group: OR (95% CI) 2.24 (1.23–4.08) ($P = 0.007$). The comparison for follicular rupture versus non-rupture at IUI showed significantly higher CPR with follicular rupture (12.6 versus 6.0) in the unexplained infertility group: OR (95% CI) 2.08 (1.01–4.31) ($P = 0.047$) and tendency toward a higher CPR (12.6 versus 6) in the anovulation infertility group: OR (95% CI) 2.11 (0.91–4.85) ($P = 0.071$).

As shown in Table II, cycles without evidence of ovulation (298 cycles) were randomized to double IUI (106 cycles) or single IUI (192 cycles) and showed no significant differences in CPR (9.4 versus 5.05, respectively): OR (95% CI) 1.9 (0.76–4.7) ($P = 0.22$). The inequality of the single and double IUI groups is because of variation in the number of IUI cases in the calendar days of allocation. We had no drop-outs among these cases because of the short follow-up period. Comparing double IUI (13.3%) and single IUI (2.8%) subgroups for male indications, CPR was higher with double insemination: OR (95% CI) 4.67 (0.90–24.13) ($P = 0.06$), however, the small number of cycles in the subgroup was insufficient to give significant differences (Type II error). In the non-male cycles, double and single IUI yielded similar CPRs (7.8 versus 6.6%): OR (95% CI) 1.20 (0.43–3.33) ($P = 0.77$) in the whole group, and for the subgroups of anovulation 1.56 (0.31–7.75) ($P = 0.62$) and unexplained infertility 1.23 (0.29–5.08) ($P = 1$) (Table II).

Comparison of single and double IUI cycles (Table III) showed no significant differences in any of the confounding variables affecting

Table II Unruptured follicle cycles at 36 ± 2 h randomized to single versus double IUI.

Parameter	All cycles	Male	Non-male		
Total unruptured	**298**		**Total**	**Anovulation**	**Unexplained**
Single IUI (*n*)[a]	192	70	122	50	72
Single IUI positive (*n*)[a]	10	2	8	4	4
Single IUI CPR[b] (%)	5.05	2.8	6.6	8	5.5
Double IUI (*n*)[a]	106	30	76	16	60
Double IU positive (*n*)	10	4	6	2	4
Double IUI CPR[b] (%)	9.4	13.3	7.8	12.5	6.6
OR (95% CI) of pregnancy in double versus single IUI	1.90 (0.76–4.70)	4.66 (0.90–24.13)	1.20 (0.43–3.33)	1.56 (0.31–7.75)	1.23 (0.29–5.08)
P	0.22[c]	0.064[c]	0.779[c]	0.626[c]	1[c]

[a]Number.
[b]Cycle pregnancy rate.
[c]χ^2 test.

Table III Comparison of single and double insemination cycles: all etiologies.

Parameter	Single IUI (*n* = 192)	Double IUI (*n* = 106)	*P*
Female age (yr)[a] (M ± SD)[b] (range)	27.58 ± 5.8 (17–45)	27.53 ± 5.7 (19–43)	0.94[c]
Infertility duration (yr)[a] (M ± SD)[b] (range)	5.09 ± 3.8 (1–19)	4.51 ± 3.6 (1–16)	0.18[c]
CC-protocol (%)	20	22	0.82[d]
CC–hMG protocol (%)	40.6	41.8	0.9[d]
hMG stimulation (%)	39.4	36.2	0.63[d]
Number of follicles >16 mm) (M ± SD)[c] (range)	2.2 ± 0.9 (1–4)	2.1 ± 0.8 (1–4)	0.381[c], $t = 0.99$, df = 296
Endometrial thickness (mm)	9.1 ± 2.5	9.4 ± 2.3	0.9021, $t = 0.123$, df = 296
Male indications (%)	33.3	25.8	0.06[d]
Fresh semen count (10^6/ml) (M ± SD)[c] (range)	53.16 ± 33.5 (1–120)	55.89 ± 32.25 (3–130)	0.9[c], $t = -0.21$, df = 296
Fresh progressive motility (%) (M ± SD)[b] (range)	53.19 ± 11.17 (5–70)	55.34 ± 7.9 (30–70)	0.08[c], $t = -1.8$, df = 296
Fresh abnormal morphology (%) (M ± SD)[b] (range)	65.6 ± 11.1 (40–95)	68.2 ± 12.4 (40–95)	0.06[c], $t = 1.8$, df = 296
IMC[e] (million/ml) (M ± SD)[b] (range)	26.84 ± 24.47 (0.5–150)	24.26 ± 18.43 (0.5–100)	0.326[c], $t = 0.98$, df = 296

[a]Year.
[b]Mean ± standard deviation.
[c]Two-tailed *t*-test.
[d]χ^2 test.
[e]Inseminated motile count.

the cycle outcome: female age, infertility duration, stimulation protocol, number of follicles, endometrial thickness or semen parameters. Even in the male subgroup, where double IUI tended to have a significantly higher CPR, the above variables did not show significant differences between double and single IUI cycles (Table IV). This indicates that any differences in the CPR are genuinely due to difference in insemination frequency and not any confounding variables. Also as shown in Table V, the incidences of multiple pregnancy and miscarriage were not significantly different among the three stimulation protocols. Figure 1 shows the distribution of IUI cycles by diagnosis (male versus non-male), ovulatory status at insemination (pre-ovulatory versus post-ovulatory) and the number of insemination (single versus double).

Discussion

The rationale of IUI treatment is to increase the rate of conception in the couple by increasing the chance that the maximum number of healthy sperm reaches the site of fertilization (The ESHRE Capri Workshop Group, 2009). Insemination can be done at various time points around ovulation and can be done once or several times. In the majority of the published studies, the insemination is done 32–36 h following hCG administration (The ESHRE Capri Workshop Group, 2009) and it is believed that IUI at 32–38 h post-hCG would provide the best results (Ragni *et al.*, 2004). The scientific basis for 32–38 h timing is derived from ultrasonographic and hormonal studies that have shown the occurrence of follicle rupture by Day

Table IV Comparison of single and double insemination cycles: male etiology.

Parameter	Single IUI ($n = 70$)	Double IUI ($n = 30$)	P
Female age (yr)[a] (M ± SD)[b] (range)	27.9 ± 6.8 (20–45)	28.06 ± 6.5 (20–43)	0.91[c], $t = 0.1092$, df = 98
Infertility duration (yr)[a] (M ± SD)[b] (range)	4.9 ± 4.2 (1–19)	4.7 ± 4.2 (2–16)	0.82[c], $t = 0.21$, df = 98
Primary infertility (%)	82.5	80	0.85[d]
CC-protocol (%)	19	22	0.75[d]
CC–hMG protocol (%)	52.5	44.7	0.52[d]
hMG stimulation (%)	28.5	33.3	0.64[d]
Number of follicles >16 mm) (M ± SD)[b] (range)	2.1 ± 0.9 (1–4)	2.1 ± 0.8 (1–4)	1[c], $t = 0$, df = 98
Endometrial thickness (mm) (M ± SD)[b] (range)	9.5 ± 2.3 (7–12)	9.1 ± 3 (8–11)	0.470[c], $t = 0.72$, df = 98
Fresh semen count (10^6) (M ± SD)[b] (range)	36.28 ± 28.7 (1–100)	41 ± 33.05 (6–100)	0.23[c], $t = -0.7$, df = 98
Fresh progressive motility (%) (M ± SD)[b] (range)	37 ± 17.1 (5–70)	43.2 ± 11.2 (20–60)	0.07[c], $t = 1.81$, df = 98
Fresh abnormal morphology (%) (M ± SD)[b] (range)	68.7 ± 12 (45–95)	73.6 ± 14 (55–90)	0.08[c], $t = 1.8$, df = 98
IMC[e] (10^6/ml) (M ± SD)[b] (range)	22.6 ± 33.3 (2–100)	12.32 ± 15.07 (0.5–40)	0.11[c], $t = 1.59$, df = 98

[a]Year.
[b]Mean ± standard deviation.
[c]Two-tailed *t*-test.
[d]χ^2 test.
[e]Inseminated motile count.

Table V First trimester outcome data related to stimulation protocols used.

	All protocols ($n^a = 1146$)	CC ($n^a = 278$)	CC–hMG ($n^a = 458$)	hMG ($n^a = 410$)	P
All pregnancies, n^a (%)	116 (10.1)	30 (10.7)	40 (8.7)	46 (11.2)	0.44[b]
Multiple pregnancies, n^a (%)	10 (8.6)	2 (6.6)	4 (10.0)	4 (8.7)	0.88[b]
Miscarriage, n^a (%)	10/97 (10.3)	2/20 (10)	4/32 (12.5)	4/46 (8.7)	0.84[b]

[a]Number.
[b]χ^2 test.

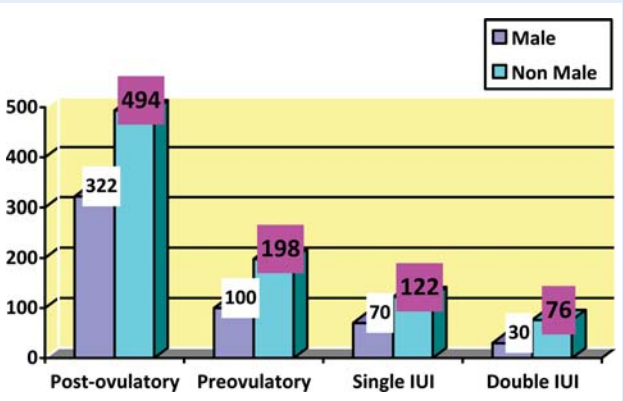

Figure 1 Distribution of IUI cycles by diagnosis, ovulatory status and number of inseminations.

+2 post-hCG in 68% of cases in spontaneous cycles (Luciano *et al.*, 1990) and in 81% (95% CI 76–86) in stimulated cycles (Pearlstone and Surrey, 1994). As shown in this study (Table I), the incidence of follicular rupture shown by TVS at the time of IUI ranged between 68 and 76%, which agrees with the above studies and lends credence to choosing the timing of IUI at 36 ± 2 h post-hCG. The overall CPR in this cohort (10.1%) is in agreement with many studies (e.g. Ragni *et al.*, 2004; Bagis *et al.*, 2010). However, different studies have reported variable overall CPRs ranging from 4.5 to 22% in stimulated cycles for different etiologies of infertility (van Rumste *et al.*, 2008). For male infertility, pregnancy rates range between 3 and 13% per cycle (Wainer *et al.*, 2004) and 11.1 and 50% per couple (Hauser *et al.*, 2001), while for anovulatory and unexplained infertility, CPRs of 36 and 24.2%, respectively, are reported (Ahinko-Hakamaa *et al.*, 2007). We feel that among the reasons for the conflicts between different studies regarding CPRs in single and double IUI is that single and double IUI were not compared in the same infertility etiologies and were not related to the occurrence of ovulation at the time of IUI. In our study, the overall CPR was significantly higher when ovulation occurred before IUI (11.7%) than when it did not (6.7%) OR (95% CI) 1.85 (1.12–3.06) ($P = 0.015$). However, when comparing CPR after and before ovulation in non-male and male factor groups, the CPR was significantly higher after ovulation with non-male factors OR (95% CI) 2.2 (1.23–4.09) ($P = 0.007$) and not so in the male factor group 1.26 (0.52–2.95) ($P = 0.82$). In both subgroups of non-male etiology (anovulation and unexplained infertility), the

difference was either significant (unexplained) or tended to be significant (anovulation; Type II statistical error). In a retrospective cohort study, Kucuk (2008) showed significantly higher CPR after follicular rupture 36–38 h post-hCG compared with that when follicle rupture was not evident. That study contained mainly couples with unexplained infertility with only 12 couples with male factor out of 417 couples with no attempt to relate CPR to the infertility etiology. The explanation for the higher CPR when IUI is timed after follicular rupture in non-male factors but not in male infertility etiology is that in the presence of fertile semen (non-male etiologies), the only limiting factor is oocyte availability which has limited survival (Edwards and Brody, 1995). Evidence of follicular rupture before IUI guarantees oocyte availability. On the other hand, in male infertility, some or all semen parameters are defective, therefore oocyte availability is not the only limiting factor. It can be rightly argued that the difference in CPR when ovulation is present and when it is absent at the time of IUI can be explained at least partially by the luteinized unruptured follicle (LUF) syndrome, which interferes with conception (Qublan *et al*., 2006; Ghanem *et al*., 2009). The CC protocol was shown to be associated with LUF in 25% of first cycles in unexplained infertility (Qublan *et al*., 2006) and in 15% of first cycles in PCOS by our group (Ghanem *et al*., 2009). The use of CC stimulation was similar in male and non-male factor cycles (20 and 22%, respectively, from our raw data not shown in results). Therefore, LUF cannot explain the differences between etiologic groups as regards CPR in ruptured and unruptured follicle cycles. Although we checked for ovulation at a repeat IUI 24 h later when we found over 80% incidence of follicular rupture, we did not verify how much of the unruptured follicles at 36 ± 2 h persisted as LUF. For exact diagnosis of the presence of LUF, cases should be monitored by TVS in the midluteal phase (7 days post-hCG trigger of ovulation; Ghanem *et al*., 2009), which is practically difficult and unethical in the IUI setting. However, considering that CC was used exclusively in only 20–22% of cycles, while sequential CC–hMG and hMG with significantly lower risk of LUF (6.9%), compared with CC only (LUF risk 15.1%; Ghanem *et al*., 2009), we can conclude that the role of LUF in our study is minimal. Another important factor to consider is that our study included analysis of only the first-treatment cycles, while Kucuk (2008) included up to three repeated cycles. It has been shown that in unexplained infertility, repeated CC cycle stimulation leads to increased incidence of LUF up to 58% in the third cycle (Qublan *et al*., 2006). Furthermore, it was shown that ideally, when evaluating therapeutic efficacy in subfertility when treatment is undertaken on a per-cycle basis, the subjects who are enrolled should be receiving treatment for the very first time. This approach of using the first cycle of treatment reduces any potential bias that may result from the experience of treatment in a previous cycle (Daya, 2003). Also the inclusion of multiple cycles from some patients introduces bias by loss of independence of data. Thus, the variables of non-pregnant subjects in multiple cycles are considered repetitively, each with the same weight as a variable from a single cycle of a pregnant patient. This practice leads to an overestimate of the importance of a single-variable predictive value (Van Voorhis *et al*., 2001).

Several retrospective studies have provided conflicting results with regard to the impact of the number of inseminations on CPR. However, meta-analysis of all cycles of single and double IUI in three randomized trials with the husband's sperm seemed to show an increased probability of pregnancy with double IUI (Duran *et al*., 2002). On the other hand, two systematic reviews found no difference in the pregnancy rate per couple with two inseminations when compared with one (Duran *et al*., 2002; Cantineau *et al*., 2003). Moreover, a more recent randomized trial of single versus double IUI in multifollicular ovarian hyperstimulation cycles in unexplained and mild male infertility (Bagis *et al*., 2010) found no difference. The last trial, however, was underpowered and included only mild male factor infertility with much higher cut-off values for sperm count and motility for male infertility (count $\geq 10 \times 10^6$, motility $<50\%$ within 1 h). The new lower reference limits of WHO for human semen (Cooper *et al*., 2009) regarding progressive motility 32(95% CI 31–34) are lower than the cut-off value of motility set by Bagis *et al*. (2010). The last study did not report on cut-off values for morphology but apparently followed the WHO criteria and not strict criteria. Thus, their infertile cases classified as male infertility by subnormal motility and morphology are not correctly classified according to this new WHO reference. Our threshold values for semen concentration and morphology are much lower as mentioned in Materials and Methods section. In our study, the minimal semen parameters in the pregnant cycles in the male factor group was ejaculate count 2×10^6/ml, progressive motility 10%, normal form 10% (strict criteria) post-processing count 0.5×10^6/ml (from raw data and not shown in results). Although we followed the 1999 WHO reference for motility assessment, the lower limits in our cases are closer to the new WHO (Cooper *et al*., 2009) lower reference limits regarding motility and morphology. These differences in the lower limits of semen parameters in our study and that by Bagis *et al*. (2010) may explain the difference in CPR with single and double IUI in male factor infertility in the two studies. Bagis *et al*. (2010) included many cases with 'normal' semen in the male factor group hence the lack of significant difference in the same way as unexplained infertility. Furthermore, they did not study temporal associations of IUI and ovulation, and as about 75% of cases are usually post-ovulatory at the time of single IUI at 36 h and second IUI at 40 h post-hCG, then a second IUI in male under these conditions will have no benefit.

Guzick (2004) and Abdelkader and Yeh (2009) reviewed the available evidence for single and double IUI and both agreed that double IUI adds more to cost and inconvenience to CPR and recommended a well-timed single IUI. However, we should remember that there are limitations to the use of meta-analysis in the context of IUI studies because of heterogeneity of the published trials (Cantineau *et al*., 2003). The studies vary in the etiologic groups and in the stimulation protocols and in the timing of insemination (Ragni *et al*., 2004). These reservations undermine the reliability of the conclusions based on meta-analysis regarding the number of inseminations per cycle. Despite these reservations and from the premise that single IUI is one of the choice, we embarked on this prospective cohort study choosing optimal timing for IUI. As already mentioned, we found that single IUI timed after ovulation in non-male (anovulation and unexplained infertility), but not in male, indications is superior when compared with that before ovulation, and that double insemination in the latter condition (absent ovulation at IUI 36 ± 2 h) tends to significantly improve CPR in male but not in female indications. The single study that related cycle outcome to IUI timing in relation to follicular rupture (Kucuk, 2008) did not compare different infertility factors in this respect. Although double IUI compared with single IUI in our

study did not increase CPR in all infertility etiologies (agreeing with most published studies), there was a trend towards a significant increase in CPR in cases of male factor infertility (13.3 versus 2.8%) OR (95% CI) 4.66 (0.90–24.13) ($P = 0.06$). Though statistically insignificant, the small subgroup numbers (30, and 70 for double and, single, respectively) point to Type 2 statistical error.

We have to acknowledge the limitations of this pilot quasi-randomized trial comparing single and double IUI in pre-ovulatory cases in male infertility. Using the observed difference in pregnancy rates, the sample size calculation (for beta = 0.80, alpha = 0.05) showed that we need a sample size of 300 cycles without follicular rupture in male subgroup to be randomized to single (200) and double (100) IUI. This means that we have to start with 900 male cycles in the whole cohort indicating the recruitment of more than 2000 cycles for the whole study to reach statistical significance, which is too much for a single center study. Again the double IUI did not increase CPR in non-male etiologies although in 80% of repeat IUI, follicular rupture was present. The variables known to predict cycle outcome such as female age, duration of infertility, type of ovarian stimulation protocol, number of follicles, endometrial thickness and fresh and post-processing semen features were all equally distributed in single and double IUI in all etiologies and in male subgroup. In Tables III and IV, it may well be observed that a tendency to better fresh progressive motility in double IUI is balanced by a tendency to better morphology in single IUI. Therefore, the differences in CPR between double and single IUI are not caused by any of the confounding variables affecting cycle outcome, particularly motility and morphology. One further possible limitation of this study is that we classified semen as subfertile if there was deviation from normal in one or more semen parameters complying with the WHO definition of infertile male (Tournaye, 2006). Definitely, abnormality only in single parameter is less detrimental to the cycle outcome compared with multiple parameter abnormalities. Most of the studies agree that semen parameters predictive of pregnancy are percentage motility and morphology (e.g. Gauci *et al*., 2001; Hauser *et al*., 2001; Guven *et al*., 2008) though using multivariate stepwise logistic regression to evaluate the weight of each sperm parameter (concentration, motility, degree of motility and morphology) in predicting pregnancy, showed that the raw semen motility percentage is most influential. The addition of other parameters did not enhance the power of prediction. In this study, it would have been more informative if male factor cycles were stratified according to the number and type of semen abnormality. However, this may be the subject of another study.

In conclusion, although single IUI in non-male factor infertility timed after follicular rupture gives better cycle outcome than before follicular rupture, double IUI in the latter cases does not significantly improve CPR. On the other hand, in male infertility, although post-ovulatory single IUI did not improve CPR significantly compared with pre-ovulatory IUI, repeating IUI in the latter case shows a trend towards significantly better CPR. Therefore, according to our results, single IUI timed after ovulation in non-male infertility is adequate with no need for double IUI in pre-ovulatory cases. In male factor, single IUI timed after ovulation is adequate, while a repeat IUI in pre-ovulatory cases tends to improve CPR. This is the first study, up to our knowledge, to study in one cohort the effect of IUI timing in relation to ovulation and to compare single and double IUI in common infertility etiologies. A larger prospective randomized trial of double versus single IUI in male factor infertiity with controlled timing in relation to ovulation is needed to validate the observed trends. Thus, minimization in effort and cost, and maximization of outcome can be made by selective use of single IUI timed after ovulation in non-male factors and double IUI in male factor if ovulation is not present at the first IUI.

Authors' roles

M.E.G.: the design, data analysis and writing of the work. N.I.B.: evaluation of semen parameters. M.A.E.: patient follow-up. L.A.A.B.: revision of the paper. A.S.H., A.G.E., M.H., I.A.A., M.M.E.: cycle monitoring and data collection.

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