SHORT COMMUNICATION

CHANGSHA, CHINA

Comparing the pregnancy rates of one versus two intrauterine inseminations (IUIs) in male factor and idiopathic infertility¹

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Purpose: To compare the effectiveness of double IUI with single IUI in male factor and idiopathic infertility patients undergoing controlled ovarian hyperstimulation (COH).

Methods: A prospective randomized study of 1257 COH-IUI cycles was performed. Total 1270 patients with mild or moderate male factor infertility ($n = 776$) and idiopathic infertility ($n = 494$) were enrolled in this study, and 767 with male factor infertility and 490 with idiopathic infertility completed clomiphene citrate (CC)/human menopausal gonadotrophin (hMG) COH cycles and IUI. Categorized on the basis of the causes of infertility (male factor, M; idiopathic infertility, I), patients were randomized into one of the following groups: Single IUI group (M_1/I_1) received single insemination 34 h post hCG administration, and double IUI group (M_2/I_2) received two inseminations 18–24 and 36–48 h post hCG injection, respectively.

Results: The overall pregnancy rates were 15.43%. Pregnancy rate for single and double IUI group was 11.06 and 19.87% ($p < 0.05$), respectively. There was a significant statistical difference in cycle fecundity between M_1 and M_2 group (11.34% vs. 24.93%, $p < 0.05$), and between I_2 and M_2 group (11.93% vs. 24.93%, $p < 0.05$), but there was no significant difference between I_1 and I_2 (10.53% vs. 11.93%, $p > 0.05$).

Conclusions: Double IUI increases the pregnancy rate significantly in patients with male factor infertility, and single IUI acts as efficient as double IUI in patients with idiopathic infertility.

KEY WORDS: Intrauterine insemination; male factor infertility; idiopathic infertility; prospective randomized study.

¹ Part of the data have been orally presented at the 21st Annual Meeting of the European Society of Human Reproduction and Embryology in Copenhagen, 2005.

INTRODUCTION

Intrauterine insemination (IUI) is a first-line assisted reproductive technique for various infertile disorders commonly utilized for many years (1). It is demonstrated that the pregnancy rate improves greatly while IUI is performed with controlled ovarian hyperstimulation (COH-IUI). Ovulation may be induced with clomiphene citrate (CC) and/or gonadotrophins. Now it is widely accepted that COH-IUI is a cost-effective way for several fertility disorders including cervical factor, mild or moderate male factor infertility and idiopathic infertility (2–5).

Though COH-IUI is a widely-used simple procedure, the efficiency of the treatment demonstrated by different clinics differs significantly; thus, there still exists many aspects that need to be modified to improve the efficiency of the treatment. Among the factors discussed, the optimal timing and number of inseminations are the hottest issues of the controversy (6–12). Intervals between hCG injection and IUI were reported anywhere between 12 and 60 h in various clinics (13), but the argument concerning the optimal frequency of insemination mostly focused on the efficiency of single and double IUI.

In 1999, Ragni *et al.* reported their data on the efficacy of double IUI versus single IUI and demonstrated that the two inseminations were the most cost-effective regime for women undergoing COH-IUI cycles because of male factor and unexplained infertility (11). But recently, Osuna *et al.* performed a meta-analysis and demonstrated that single IUI was as efficient as double IUI (6). We proposed that best efficiency of the treatment could be achieved via different COH-IUI regimens being introduced to patients with different causes of infertility.

The aim of our randomized, prospective study was to compare the efficiency of single COH-IUI versus double COH-IUI in patients with mild or moderate male factor and idiopathic infertility respectively.

MATERIALS AND METHODS

Patients

This study was approved by the Hospital Ethical Committee of Central South University in accordance with Helsinki Declaration of 1975 on human experimentation. Written informed consent was obtained from each patient involved. A total number of 1270 patients with mild or moderate male factor

infertility ($n = 776$) and idiopathic infertility ($n = 494$) were enrolled for a single cycle of COH-IUI. Seven hundred and sixty-seven patients with male factor infertility and 490 with idiopathic infertility completed the treatment cycle. All couples had a history of infertility at least 24 months.

All patients had a complete infertility workup consisting of a medical history, basal body temperature (BBT), ultrasonography, hysterosalpinograph (HSG), basal hormonal test (FSH, LH, E_2 , PRL, T_3 , T_4 and T on day 3 of menstruation and P on day 25), semen analysis, postcoital test (PCT) and, in some patients, endometrial biopsy and diagnostic laparoscopy. Male factor infertility was diagnosed when at least two semen analysis showed subnormal according to the World Health Organization criteria (14): in brief, mild male infertility is defined when sperm count is between 15 and $20 \times 10^6/\text{mL}$, motility ranges from 40 to 50%, normal morphology 30–40%; moderate male infertility is a male with a count between 10 and $15 \times 10^6/\text{mL}$, motility range 20–40% and/or normal morphology 10–30%. Idiopathic infertility was defined as couples with infertility history of at least 24 months and with normal results in the aforementioned tests including endometrial biopsy and diagnostic laparoscopy. Patients with an- or oligo-ovulation, tubal diseases, endometriosis, cervical factor and PCOS were excluded. All patients underwent their first cycle of IUI treatment.

Ovulation Induction

All patients underwent controlled ovarian hyperstimulation (COH) cycles. Hyperstimulation was performed by starting with or without 50 mg per day citrate clomiphene for 5 days from day 3 to 7 of menstrual cycles. Human menopausal gonadotrophin (hMG) was given on days 5, 7, and 9 at a dosage of 75–150 IU daily according to the body weight. Follicle development was monitored by consecutive transvaginal ultrasonography starting on day 10 and the dosage of hMG was adjusted according to ovarian response. Serum E_2 was examined when the diameter of a follicle reached 16 mm (it is defined as a dominant follicle while its diameter reached 16 mm). When the diameter of dominant follicle ≥ 18 mm and the serum E_2 level reached a level of average 200 pg/mL/dominant follicle, a single dose of 10,000 IU human chorionic gonadotrophin (hCG) was administered to trigger ovulation. There were no cases of severe ovarian hyperstimulation syndrome.

Sperm Preparation, IUI, and Pregnancy Confirmation

The husbands were asked to be abstinent for 3–5 days before semen collection. Semen specimens were obtained by masturbation into a sterile jar a few hours before the scheduled time of insemination in our clinic or at home and prepared in our clinic according to standard WHO procedures (14). In brief, the samples were placed in 37°C incubator for 15–30 min to liquefy. The entire semen sample was washed once by Ham's F-10 medium (Sigma, St. Louis, MO), then prepared by centrifugation on a two-layer Percoll density gradient. After wash by Ham's F-10 medium once more, the pellet was resuspended in 0.5 mL medium and used immediately for insemination.

Matched for ages and duration of infertility within the same etiologic cause group, patients with male factor infertility (M) and idiopathic infertility (I) were separately randomized into single or double IUI group (M1, M2; I1 and I2) using random number table, group. Only one insemination was performed at 34 h post HCG injection in single IUI group; and semens were inseminated twice at 18–24 and 36–48 h post HCG injection in double IUI group, respectively. IUI was accomplished by inserting the catheter into the uterine cavity near the fundus and slowly injecting 0.5 mL prepared sperm inside. Women had 30 min to 1 h bed rest after IUI.

Clinical pregnancy was confirmed by the detection of fetal heart beats by transvaginal ultrasonography examination at 6 week gestation age, and ongoing pregnancy was defined by the ultrasonography examination at 12 week gestation age.

Statistical Analysis

Categorical data between the groups were compared with the χ^2 -test and t -test. Non-parametric data were compared with Wilcoxon test and Kruskal–Wallis H -test. Data were analyzed by Statistical Package for the Social Science computer program (SPSS 12.0/Windows; SPSS Inc., Chicago, IL).

RESULTS

One thousand two hundred and fifty-seven of the 1270 recruited patients had finished the assigned treatment cycles, and 13 of them dropped out because of various reasons; thus, there were 386, 381,

Table I. The Number ($\times 10^6$) of Motile Spermatozoa Inseminated (Median, Range)

	One IUI	First IUI	Second IUI	<i>p</i> -value ^b
Idiopathic infertility	39 (10–53)	36 (11–47)	24 (10–29)	0.0978
Male factor infertility	16 (5–27)	15 (4–31)	9 (2–27)	0.1361
<i>p</i> -value ^a	0.001334	0.00371	0.04613	

^aSignificance between male factor and idiopathic infertility, *p*-value obtained by Wilcoxon test.

^bNo significance between one and two IUI groups with the same cause of infertility, *p*-value by Kruskal–Wallis *H*-test.

247, and 241 patients in M₁, M₂, I₁, and I₂ groups, respectively. The average maternal age and infertility duration of patients with male factor infertility were 32.1 ± 3.3 and 3.4 ± 2.7 and those with idiopathic infertility it were 34.9 ± 3.5 and 4.9 ± 5.2 . There were no significant statistical differences between the groups with respect to the maternal age and duration of infertility. The number of motile spermatozoa [a + b, refers to the WHO category of sperm motility and progression; 'a' refers to rapid progressive motility and 'b' to slow or sluggish motility (15)] inseminated for patients with male factor infertility was less than those with idiopathic infertility ($p < 0.05$), but for patients with the same cause of infertility, differences in the number of motile spermatozoa inseminated between groups were not statistically significant ($p > 0.05$) (Table I). On the day of hCG injection, the mean number of dominant follicles were 3.04 ± 1.21 and 2.98 ± 1.05 in M₁ and M₂ group, and 3.41 ± 1.14 and 3.12 ± 1.37 in I₁ and I₂ group, respectively. There were no significantly statistical differences between the groups ($p > 0.05$).

Clinical pregnancy rate was calculated and compared in this study. One hundred and ninety-four patients achieved a pregnancy altogether (194/1257, 15.43%). Pregnancy rate was 11.06% in single IUI cycle and 19.87% in double IUI cycle; the rate had significant statistical differences ($p < 0.05$). Overall, 55 idiopathic infertility patients (11.22%) and 139 male factor infertility patients (18.12%) achieved

pregnancy ($p < 0.05$). Forty-four pregnancies were achieved in M₁ group (11.34%), 95 in M₂ group (24.93%), 26 in I₁ group (10.53%), and 29 in I₂ group (11.93%). There was a significant statistical difference between M₁ and M₂ ($p < 0.05$) and between I₂ and I₁ ($p < 0.05$) as well. That means the pregnancy rate for male factor infertility in double IUI group was higher than that in single IUI group, and for idiopathic infertility, there was no significant difference between single and double IUI groups (Table II).

Overall, there were four twins (one in M₁ and three in M₂) in male factor group and two in I₂ group; only one triplet seen in M₂ group. And 11 abortions occurred (two in M₁, five in M₂; one in I₁, and three in I₂ group).

DISCUSSION

IUI in controlled hyperstimulation cycles (COH-IUI) is an extremely common treatment for a wide variety of infertility problems and because of its relatively low cost and less invasion, it is now widely used as the first-line therapy before more expensive and invasive assisted reproductive techniques, such as IVF or GIFT, to treat those infertile couples with patent tubes (10). As data were limited and varied among different studies because of the heterogeneity in patient population and time and number of inseminations performed (16), studies on

Table II. Comparison of the Pregnancy Rates (%) Between Groups

	One IUI	Two IUIs	<i>p</i> -value ^a
Idiopathic infertility	10.53% (26/247)	11.93% (29/243)	0.6806
Male factor infertility	11.34% (44/386)	24.93% (95/381)	0.000106
<i>p</i> -value ^b	0.7474	0.0017	
Overall pregnancy	11.06% (70/633)	19.87% (124/624)	0.0027

^aSignificance between one versus two IUI. *p*-value obtained by χ^2 -test.

^bSignificance between male factor and idiopathic infertility. *p*-value obtained by χ^2 -test.

clarifying the factors that affect the pregnancy rate would still be helpful.

In our study, the overall pregnancy rate was 15.43%, among which 94.33% (183/194) were ongoing. Our data were comparable to the pregnancy rates of other studies in which the clinical pregnancy rate ranged from 9.2 to 33.3% (1,10–13,17).

In this prospective randomized study, we categorized patients into different etiological group and compared the efficacy of double IUI with single IUI in patients with male factor and idiopathic infertility separately. The overall pregnancy rate for idiopathic infertility was 11.22% per cycle, while for male factor infertility it was 18.12% per cycle. The pregnancy rate per cycle for male factor infertility was significantly higher than that for idiopathic infertility ($p < 0.05$). These findings were not exactly similar to Alborzi's result (10). According to his report, the pregnancy rate for idiopathic infertility and male factor infertility was 7.88 and 6.38% per cycle, ours was higher than his. Also, they got much similar pregnancy rate for both the causes and in our study, the cycle pregnancy rate was higher for male factor infertility. The reason might be that they included all male factor infertility, but we only studied those with mild and moderate male infertility according to WHO criteria. In our study, those patients with sperm count in the range $10\text{--}20 \times 10^6/\text{mL}$ and/or motility of 20–50%, and/or 10–40% normal morphology were recruited. The patients were excluded if the male partner had sperm parameters worse than the range. Also, we only analyzed those patients who underwent their first cycle of treatment. There is a tendency that with the increase in the number of cycles, the pregnancy rate per patient would increase, while the pregnancy rate per cycle would decrease. Though more motile sperms reach the fertilizing site counts for better result after IUI, there might be some undetectable minor reproductive defects that had negative effects on the fecundity of unexplained infertility patients.

The number of insemination performed was still controversial. In Osuna's systematic review (6), the reason supporting one insemination was that the fertilization window for oocytes was 12–24 h and the natural fertilizing capacity could be as long as 48–72 h after ejection to vagina. Only one insemination in the appropriate time should be theoretically enough for fertilization. Also, 'in cases with poor sperm the second sperm sample could be of inferior quality and might not provide benefit with double IUI' (6). But, in our study, the double IUI for male factor infertility was the winner. Part of the reason might be that the

second insemination was performed 24 h later from the first one, thus the motile sperm count in the second sample was still 9×10^6 ($2\text{--}27 \times 10^6$) and even the lowest number reached the criteria for IUI which will get a reasonable pregnancy rate (1). We would like to speculate that motile sperm count accounts most for the fecundity of the male factor infertility, the second insemination just adds up the motile sperm number for fertilization and also served as a cervical crypt and releases the motile sperm for those late ovulated oocytes in a COH cycle and thus makes the most of 'the window of opportunity for IUI'.

In conclusion, our data suggested that the frequency of IUI might better be determined according to the specific etiological causes of infertility; for patients with male factor infertility, double IUI would increase the clinical pregnancy rate significantly and one well-timed IUI seemed a better choice for those with idiopathic infertility.

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REFERENCES

1. Iberico G, Vioque J, Ariza N, Lozano JM, Roca M, Liacer J, Bernabeu R: Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. *Fertil Steril* 2004;81:1308–1313
2. Silverberg KM, Johnson JV, Olive DL, Burns WN, Schenken RS: A prospective, randomized trial comparing two different intrauterine insemination regimens in controlled ovarian hyperstimulation cycles. *Fertil Steril* 1992;57:357–361
3. Dodson WC, Haney FF: Controlled ovarian hyperstimulation and intrauterine insemination for treatment of infertility. *Fertil Steril* 1995;55:457–467
4. Hughes EG: The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: A meta-analysis. *Hum Reprod* 1997;12:1865–1872
5. Guzick DS, Carson SA, Coutifaris C, Overstreet JW, Factor-Litvak P, Steinkampf MP, *et al.*: Efficacy of superovulation and intrauterine insemination in the treatment of infertility. *N Engl J Med* 1999;340:177–183
6. Osuna C, Matorras R, Pijoan JI, Rodriguez-Escudero FJ: One versus two inseminations per cycle in intrauterine insemination with sperm from patients' husbands: A systematic review of the literature. *Fertil Steril* 2004;82:17–24
7. Ragni G, Somigliana E, Vegetti W: Timing of intrauterine insemination: Where are we? *Fertil Steril* 2004;82:25–26
8. Guzick DS: For now, one well-timed intrauterine insemination is the way to go. *Fertil Steril* 2004;82:30–31

9. Cantineau AEP, Heineman MJ, Cohlen BJ: Single versus double insemination in stimulated cycles for subfertile couples: Systematic review based on a Cochrane review. *Hum Reprod* 2003;18:941–946
10. Alborzi S, Motazedian S, Parsanezhad ME, Jannati S: Comparison of the effectiveness of single intrauterine insemination (IUI) versus double IUI per cycle in infertile patients. *Fertil Steril* 2003;80:595–599
11. Ragni G, Maggioni P, Guermandi E, Testa A, Baroni E, Colombo M, *et al.*: Efficacy of double intrauterine insemination in controlled ovarian hyperstimulation cycles. *Fertil Steril* 1999;72:619–622
12. Madankumar R, Tsang J, Lesser ML, Kenigsberg D, Brenner S: Clomiphene citrate induced ovulation and intrauterine insemination: Effect of timing of human chorionic gonadotropin injection in relation to the spontaneous LH surge on pregnancy rates. *J Assist Reprod Genet* 2005;22:155–159
13. Claman P, Wilkie V, Collins D: Timing intrauterine insemination either 33 or 39 h after administration of human chorionic gonadotropin yields the same pregnancy rates after superovulation therapy. *Fertil Steril* 2004;82:13–16
14. World Health Organization: Collection and examination of human semen. In *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, 4th edn., Cambridge, Cambridge University Press, 1999; pp. 4–5
15. World Health Organization: *WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 3rd edn., Cambridge, Cambridge University Press
16. Osuna C, Matorras R, Pijoan JI, Rodriguez-Escudero F: The heterogeneity of randomized, controlled trials of intrauterine insemination. *Fertil Steril* 2004;83:34–35
17. Sahakyan M, Harlow BL, Hornstein MD: Influence of age, diagnosis, and cycle number on pregnancy rates with ganadotrophin-induced controlled ovarian hyperstimulation and intrauterine insemination. *Fertil Steril* 1999;72:500–504

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