CLINICAL ASSISTED REPRODUCTION

An Open Multicenter Study to Compare the Efficacy of Intraperitoneal Insemination and Intrauterine Insemination Following Multiple Follicular Development as Treatment for Unexplained Infertility[1]

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Purpose: *This multicenter study was carried out to compare the efficacy of intrauterine insemination (IUI) and intraperitoneal insemination (IPI) associated with multiple follicular development as treatment for unexplained infertility.*
Method: *A total of 205 couples completed the trial. Sixty-seven couples underwent treatment with IPI (group A) and 138 couples underwent treatment with IUI (group B).*
Results: *Clinical pregnancy was obtained in 23 couples in group A (pregnancy rate: 34.3%) and in 36 couples in group B (pregnancy rate: 26.1%). No significant difference was observed between group A and group B. As for the evolution of pregnancies and the incidence of twin pregnancies, no significant difference was observed between the two groups.*
Conclusions: *Because IUI and IPI allow us to obtain same results and IPI is more invasive than IUI, the latter technique can be considered the method of choice and IPI should be used when IUI is difficult to perform, as in the presence of a tight cervical canal.*

KEY WORDS: intraperitoneal insemination; intrauterine insemination; multicenter study; multiple follicular development; unexplained infertility.

INTRODUCTION

For many years artificial insemination has been performed mainly depositing the semen in the vagina and/or in the cervix (1). These techniques have been replaced by sperm deposition in upper tracts of the female reproductive organs (2–4). Intrauterine insemination (IUI) simply requires injection of a small amount of prepared sperm into the uterine cavity by using a thin catheter passed through the cervix (2). Otherwise, intraperitoneal insemination (IPI) is performed by injection of prepared sperm directly in the pouch of Douglas by culdocentesis (3, 4).

Since both IUI and IPI require the same sperm preparation and are performed after induction of multiple follicular development (MFD), differences in the pregnancy rate obtained with the two techniques should depend only on the site of sperm deposition.

Until now, few studies (5–9) have been performed to compare the results obtained with the two techniques. These studies considered a small sample size or few consecutive cycles for each couple, while it has been

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reported that repeated cycles of induction of MFD associated with insemination allow reaching a pregnancy rate that is comparable to that with major techniques (10).

The aim of this study was to compare the efficacy of IPI with that of IUI as treatment for unexplained infertility in a multicenter trial.

MATERIALS AND METHODS

Patients

The study population was defined by the inclusion and exclusion criteria described below to select a homogeneous group of infertile patients.

The inclusion criteria were as follows:

(1) Female age ≤40 years.
(2) Duration of infertility ≥2 year
(3) Diagnosis of unexplained infertility [progesterone >8 ng/ml (conversion factor to SI units is 3.180) for at least 10 days and prolactin <15 ng/ml in the luteal phase of the cycle, postcoital test adequate, bilateral tubal patency, and no pelvic pathology; sperm analysis with a sperm concentration $\geq 20 \times 10^6$/ml, progressive motility ≥25%, total motility ≥50%, and normal morphology ≥50%].

The exclusion criteria were as follows.

(1) Clinically relevant systemic disease.
(2) Treatment for infertility in the previous 6 months.

All couples gave fully informed consent to participate in the study, for which local ethical committee approval was obtained.

Study Design

This clinical trial was an open, comparative study conducted at four centers. Each center enrolled 60 couples. All couples were assigned to one of the following two treatment groups: group A, three consecutive cycles of multiple follicular development followed by IPI; and group B, three consecutive cycles of multiple follicular development followed by IUI. The fertility centers that participated in the study could randomly assign the couples to one of the two groups or perform the techniques customarily used at that center.

Multiple Follicular Development

Multiple follicular development (MFD) was obtained by administering follicle-stimulating hormone (FSH; Metrodin, Serono, Rome, Italy), starting with a daily dose of 2 ampoules from the third day of the cycle. During treatment with exogenous gonadotropins, pelvic ultrasonography, to determine the number and diameter of ovarian follicles, and blood samples for estradiol (E_2) rapid assay (Medical System, Genova, Italy) were obtained every other day until the mean diameter of the dominant follicles reached 10 mm and E_2 plasma levels reached 100 pg/ml (conversion factor to SI units for E_2 is 3.671). Thereafter both examinations were performed daily. The dose of FSH was adjusted according to ultrasound picture and endocrine monitoring. The treatment was discontinued when E_2 plasma levels reached 600–1800 pg/ml and there were at least two follicles with a mean diameter ≥14 mm. Human chorionic gonadotropin (hCG, Profasi; Serono, Rome, Italy; 10,000 IU) was administered 24–36 hr after the last injection of FSH.

The administration of a GnRH analogue, starting on day 1 of the menstrual cycle, was optional.

IUI or IPI was performed 30–36 hr after hCG administration.

The cycles were canceled if no follicles were found and/or the E_2 level was ≤50 pg/ml after 50 ampoules of FSH, ovarian cysts ≥35 mm in diameter were found by ultrasound, or there was the risk of severe hyperstimulation syndrome.

Progesterone luteal support was not used in any of the patients.

Sperm Preparation

Sperm was prepared by the conventional layering technique for both IUI and IPI. Approximately 1.0 ml of medium was layered onto 1 ml of sperm and the specimen was incubated at 37°C for 60 min. At the end of the incubation period, the uppermost layer of the medium, containing motile spermatozoa, was collected. If the semen sample was too viscous, it was diluted 1:5 with medium and centrifuged for 5 min before performing the layering.

Insemination Techniques

IUI. The female partner was in the lithotomy position. The cervix was exposed with a vaginal speculum and cleaned with normal saline. The sperm suspension was drawn into a Kramer de la Fontaine catheter

attached to a 1-ml syringe and the catheter was passed into the uterus until it lay about 0.5 cm from the top of the uterine cavity in the fundal region. The in vitro prepared sperm was gently injected into the uterine cavity and the catheter was subsequently withdrawn 1 min after the injection of the sperm suspension. The patient laid in the supine position for 15 min after the insemination.

IPI. The female partner was in the lithotomy position and monitored by ultrasound. The cervix was exposed with a vaginal speculum. The posterior fornix was sprayed with a solution of 10% xylocaine. The cervix and the vagina were carefully disinfected, removing the mucus from the posterior fornix. A 19-gauge Butterfly needle was connected to a 2-ml syringe containing the sperm suspension and was inserted into the pouch of Douglas through the posterior vaginal fornix, using a needle-holder. Before injecting the sperm suspension, a light aspiration was carried out to verify that the tip of the needle was actually inserted into the pouch of Douglas. The sperm suspension was gently injected and the Butterfly needle was withdrawn 1 min after the injection. The patient laid in the supine position for 15 min after the insemination.

Pregnancy

Pregnancies were considered clinical when the elevation of serum hCG above 25 IU/L was verified by transvaginal ultrasound examination (presence of an embryonic heartbeat). Chemical pregnancies were not considered in the calculation of pregnancy rate and life table analyses.

Statistical Analysis

The following data were compared: duration of FSH administration, dose of FSH in each cycle, number of follicles on the day of hCG administration, number of follicles with a diameter $\geq$14 mm on the day of hCG administration, plasma levels of E_2 on the day of hCG administration, pregnancy rate, incidence of twin pregnancies, and abortion rate.

Life table analysis was performed in the two groups of patients and log rank test was used to compare results of life table analysis.

Chi-square and logistic regression analysis were used to compare pregnancy rates. For comparison of the means, Student's *t* test was performed whenever appropriate.

RESULTS

At the end of the multicenter trial a total of 212 couples was enrolled in the study: center 1 enrolled 60 patients and performed only IUI; both center 2 and center 3 enrolled 60 patients and randomized the patients, thus they performed 30 IPI and 30 IUI; and center 4 concluded the study when it had enrolled 32 patients and randomized 19 patients to IUI and 13 to IPI. Where performed, numbered sealed envelopes were used for randomization. Seven couples (six in group 1 and one in group 2) were excluded from the study because of failure to complete the three cycles of treatment. Thus, 205 couples completed the trial, for a total of 510 cycles. Sixty-seven couples underwent treatment with MFD associated with IPI (group A) and 138 couples underwent treatment with MFD associated with IUI (group B).

No significant difference was present between treatment groups as regards female age distribution, infertility period, age at menarche, male age distribution, and parameters of sperm analysis (Table I).

In 107 patients a GnRH analogue was used for induction of MFD, whereas in 98 patients MFD was induced without administration of GnRH analogue. Considering the overall three stimulation cycles, in the IUI group 62% of the women were administered a GnRH analogue, whereas in the IPI group 21% of the patients received a GnRH analogue.

As for the data concerning the stimulation parameters in each cycle, no significant difference was present between treatment groups regarding the duration of FSH administration, total dose of FSH, total number of follicles on day of hCG, number of follicles $\geq$14 mm in diameter on the day of hCG, and E_2 levels on day of hCG (Table I).

Clinical pregnancy was obtained in 23 couples in group A (pregnancy rate: 34.3% per patient, 15.1% per cycle) and in 36 couples in group B (pregnancy rate: 26.1% per patient, 10.2% per cycle). No significant difference was observed between group A and group B. As for the evolution of pregnancies, no significant difference was observed between the two groups. The incidence of twin pregnancies was 19% with both types of inseminations. Life table analysis is shown in Fig. 1. No significant difference was present between the groups.

Dividing the patients into two groups according to the use of GnRH agonist (independently of the type of insemination), the percentage of clinical pregnancies was 29% (31/107) in the group of patients who received GnRH analogue (GnRH + group) and 30%

Table I. Characteristics of the Couples in the Study Groups[a]

	MFD + IPI[b] (group A)	MFD + IUI[c] (group B)	*P*
Number of couples	67	138	
Female age (yr)	32.4 ± 3.19	32.0 ± 3.38	NS[d]
Infertility period (yr)	4.25 ± 2.23	4.01 ± 2.37	NS
Age at menarche (yr)	11.83 ± 1.06	12.16 ± 1.25	NS
Male age (yr)	35.8 ± 3.31	35.7 ± 4.06	NS
Sperm count (per ml)	68.38 ± 39.05 × 10^6	61.90 ± 31.85 × 10^6	NS
Progressive motility (%)	35.13 ± 12.9	44.4 ± 14.73	NS
Normal morphology (%)	59.18 ± 9.26	64.7 ± 13.02	NS
Days of FSH administration[e]	11.21 ± 2.48	11.16 ± 2.09	NS
Total dose of FSH (IU)[e]	1503.53 ± 351.96	1581.29 ± 175.83	NS
Total number of follicles[e]	8.31 ± 2.98	8.01 ± 3.26	NS
Follicles ≥14 mm in diameter[e]	5.17 ± 1.68	4.99 ± 1.8	NS
E_2 levels (pg/ml)[e,f]	1323.04 ± 392.77	1347.31 ± 378.07	NS

[a] Values are means ± SD.
[b] Multiple follicular development associated with intraperitoneal insemination.
[c] Multiple follicular development associated with intrauterine insemination.
[d] Not significant.
[e] Day of hCG administration.
[f] Conversion factor to SI units is 3.671.

(29/98) in the group of patients who did not receive GnRH analogue (GnRH − group) ($P > 0.05$). As regards the data concerning the stimulation parameters in each cycle, no significant difference was present between treatment group regarding the duration of FSH administration, total dose of FSH, total number of follicles on day of hCG, number of follicles ≥14mm in diameter on the day of hCG, and levels of E_2 on the day of hCG (Table II).

Logistic regression analysis demonstrated no significant difference among pregnancy rates obtained in patients submitted to IUI who received GnRH-a, patients submitted to IUI who do not receive GnRH-a, patients submitted to IPI who received GnRH-a, and patients submitted to IPI who do not receive GnRH-a.

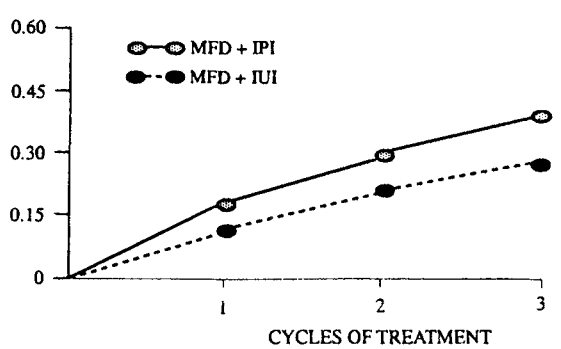

Fig. 1. Life table analysis of pregnancy in the study groups ($P > 0.05$). MFD + IPI, multiple follicular development associated with intraperitoneal insemination; MFD + IUI, multiple follicular development associated with intrauterine insemination.

No severe hyperstimulation syndrome was referred by the four centers. The side effects reported were a slight increase in weight in one case, a slight increase in weight and slight nausea in one case, and a moderate increase in weight, moderate nausea, and slight diarrhea in one case. In all cases these side effects disappeared after 2–10 days without discontinuing treatment.

DISCUSSION

The result of this multicenter, prospective study demonstrates that IUI and IPI have a comparable effectiveness in obtaining pregnancy when performed after induction of multiple follicular development in couples affected by unexplained infertility. However, although there was no significant difference between the two groups, it appears that there was a slight advantage with IPI in terms of pregnancy rates. As a matter of fact, to demonstrate a difference as significant as a 5% increase in pregnancy rate per cycle and an 8% increase in pregnancy rate per patient for IPI versus IUI, a total of 1452 cycles (726 per treatment group) and 698 patients (349 per treatment group) would need to be studied to warrant a chi-square test with a power of 80% (11).

The IPI was introduced by Mahnes and Hermabessiere (12) and Forrler *et al.* (3) as an alternative to IUI and GIFT or IVF (3,6,12), with the theoretical aim of taking advantages of the positive influence of both

Table II. Stimulation Parameters in the Two Groups of Patients[a]

	GnRH+[b]	GnRH−[c]	*P*
Days of FSH administration[d]	10.81 ± 2.16	11.53 ± 2.22	NS[e]
Total dose of FSH (IU)[d]	1544.12 ± 208.49	1570.09 ± 277.08	NS
Total number of follicles[d]	8.24 ± 3.43	8.00 ± 2.87	NS
Follicles ≥14 mm in diameter[d]	5.04 ± 1.95	5.10 ± 1.56	NS
E_2 level (pg/ml)[d,f]	1420.76 ± 361.89	1262.69 ± 386.19	NS

[a] Values are means ± SD.
[b] Patients who received GnRH analogue.
[c] Patients who did not receive GnRH analogue.
[d] Day of hCG administration.
[e] Not significant.
[f] Conversion factor to SI units is 3.671.

cumulus oophorus and follicular fluid on sperm motility and acrosome reaction (13,14), avoiding the more expensive and invasive technique. The first studies reported encouraging results (15). In fact, pregnancy rates were similar to those obtained with GIFT (16) and pregnancies were also obtained in couples who had previously undergone a different kind of treatment (4, 17).

Some years later, prospective and randomized studies comparing results obtained with IUI and IPI in small samples of women who underwent multiple follicular development did not confirm the superiority of IPI or IUI (5,6,8,9), and only Evans *et al.* (7) reported significantly higher pregnancy rates in couples submitted to IPI. All these studies considered few consecutive cycles of the same treatment for each couple.

In a larger sample of couples affected by unexplained infertility, this multicenter prospective study confirms the lack of better results with IPI when the two techniques are performed after induction of multiple follicular development. Besides, these couples underwent three consecutive cycles of the same treatment to obtain the best results, avoiding the influence of a carryover effect of multiple follicular development on the, otherwise different, successive treatment (10). Thus, unless further very large studies (approximately 700 patients with 2000 cycles) demonstrate a significant difference in pregnancy rate between the two techniques, IUI and IPI seem to produce the same results, and since IPI is more invasive than IUI, the latter technique can be considered the method of choice and IPI should be used when IUI is difficult to perform, as in the presence of a tight cervical canal.

Recent studies demonstrated that the improved pregnancy rate obtained with multiple follicular development associated with artificial insemination was attributable mostly to the use of multiple follicular development; in fact the pregnancy rates obtained with multiple follicular development associated with timed vaginal intercourse were comparable to those obtained when multiple follicular development was associated with artificial insemination (10,18). Therefore, timed intercourse, IUI, and IPI can be considered as treatment for unexplained infertility only if associated with an accurate multiple follicular development.

As for the stimulation protocol, in this study no significant difference was observed in the level of E_2 on the day of hCG administration in pregnant versus nonpregnant women (1301 ± 419.49 versus 1343 ± 368.86 pg/ml, respectively; $P > 0.05$), and the use of a gonadotropin-releasing hormone agonist, administered according to a short regimen, did not improve the pregnancy rate and did not modify the days of FSH administration, the total dose of FSH, the number of follicles, or the E_2 levels (Table II). Besides, during the trial no severe hyperstimulation was observed, and side effects definitely associated with the treatment (increase in weight, nausea, and diarrea) were reported in only three patients (1.5%) and disappeared after a few days without discontinuing the treatment. These data suggest that since GnRH agonists are expensive, these drugs should be used only in selected patients with a previous spontaneous LH surge or in patients who undergo more invasive techniques such as IVF-ET or GIFT.

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