Comparison of intrafollicular sperm injection and intrauterine insemination in the treatment of subfertility

Caterina Lucchini · Emanuele Volpe · Angelo Tocci

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Abstract

Purpose To compare the efficacy of intrafollicular sperm injection (IFI) versus intrauterine insemination (IUI) in the treatment of subfertility.

Methods 38 couples suffering primary or secondary subfertility contributed a total of 47 IUI or IFI cycles, 26 by IUI and 21 by IFI. Folliculogenesis, ovulation triggering, and IUI or IFI were performed. Motile spermatozoa were inseminated into the uterine cavity for IUI or injected into pre-ovulatory follicles for IFI. The rate of biochemical and clinical pregnancy was assessed.

Results The rate of biochemical pregnancy/cycle for IUI was 11 % as compared to 38 % for IFI ($p=0.04$). The rate of clinical pregnancy/cycle for IUI was 11 % as compared to 29 % for IFI ($p=0.26$). The rate of twin pregnancy and miscarriage was low and no high order multiple gestation was observed. The rate of ectopic tubal pregnancy/cycle for IUI was 0 % as compared to 9 % for IFI ($p=0.19$); no ovarian pregnancy was observed. When the analysis was confined to IFI cycles in which 2.68–6.65 million motile spermatozoa were injected/follicle ($n=10$), a rate of 60 % clinical pregnancy/cycle was observed, of which 2 were ectopic.

Conclusion Under the conditions described herein, IFI was more effective than IUI at achieving pregnancy.

Keywords Intrafollicular sperm injection · Intrauterine insemination

Introduction

Intrauterine insemination (IUI) and intrafollicular sperm injection (IFI) are assisted reproduction techniques (ARTs) in which fertilization takes place in the female reproductive tract.

IUI is a widely accepted treatment for mild subfertility although it has been proven modestly superior to observation, and even then, only in association with controlled ovarian hyper-stimulation (COH) [1]. The European IVF Monitoring Program reported a rate of 12 % clinical pregnancy/cycle in women below 40 years, of which 11.4 % were twin and 2.2 % were triplet gestations. The rate of biochemical, miscarried and ectopic pregnancy was not reported [2].

Earlier studies on IFI, including single case reports and the largest case series published so far, reported a rate of 13 % clinical pregnancies/cycle in women below 44 years, of which 14 % were twin, 14 % miscarried, and 3.4 % were ectopic gestations [3–6]. In most studies investigating IFI, few technical details have been provided while a thorough analysis reveals some possible pitfalls in the design, patient selection, and technical interventions. In a pilot study on 9 IFI procedures, we observed 4 biochemical and 3 clinical pregnancies [7]. However, the applicability of IFI remains to be determined and studies comparing IFI to IUI are lacking.

Our aim was to compare the efficacy of IFI versus IUI for the treatment of subfertility and provide new insights into the mechanisms of IFI.

Capsule Thirty-eight couples suffering infertility contributed 47 cycles of intrafollicular sperm injection (IFI) or intrauterine insemination (IUI). Under the conditions described herein, IFI was more effective than IUI at achieving pregnancy.

C. Lucchini · E. Volpe · A. Tocci (✉)
Gruppo Donnamed, Reproductive Medicine Unit,
Nuova Villa Claudia Clinic,
Via Flaminia Nuova 280,
Rome, Italy
e-mail: staffdonnamed@gmail.com

A. Tocci
e-mail: gruppodonnamed@gmail.com

Materials and methods

Patients

Thirty-eight couples with primary or secondary infertility participated in our program of assisted reproduction for the treatment of mild infertility, and were treated by either IUI or IFI according to the national guidelines (2008). Patients were identified from the database of the Gruppo Donnamed IVF center located in Rome, Italy, at the Nuova Villa Claudia private clinic. A detailed written informed consent to the clinical procedures was obtained from all couples. All couples agreed to a possible publication of their anonymous data. Because the study was retrospective in nature, it was not necessary to ask for Institutional Review Board approval according to the guidelines of the Commission for Medical Ethics in our clinic.

The patients contributed a total of 47 cycles. Nine of the 21 cycles of IFI were reported in a previous publication [7]. A cycle was defined as a COH, a natural or a modified natural procedure that resulted in an IUI or IFI. Women 27 through 44 years of age who fulfilled the inclusion criteria were informed in detail about the advantages and disadvantages of IUI and IFI and left free to choose their preferred treatment. Twenty patients underwent 26 IUI cycles and 18 patients underwent 21 IFI cycles. Inclusion criteria were mild male factor ($n=15$), unexplained infertility ($n=14$), anovulation in patients who failed COH and timed intercourse ($n=5$), mono-lateral tubal occlusion ($n=1$), minimal or mild endometriosis ($n=4$), cervical factor ($n=2$), or a combination of two factors ($n=1$ endometriosis and cervical factor, $n=2$ male and pelvic factor). Exclusion criteria were bilateral tubal occlusion, severe oligozoospermia, and impaired ovarian reserve.

All couples underwent fertility investigation by a medical history, physical examination, semen analysis performed according to standardized methods by trained biologists [8, 9], confirmation of ovulation, tubal patency testing, and assessment of ovarian reserve. Laparoscopy was carried out in women suspected to have endometriosis. Male subfertility was defined as semen analysis below the criteria of World Health Organization 2010 [8] for men with proven fertility or below the criteria of an unscreened general population if fertility status was unknown [9]. Unexplained subfertility was declared if no abnormality was found during fertility investigations. Tubal patency was demonstrated by hysterosonosalpingography, hysterosalpingography or laparoscopy. Ovulation was inferred by a regular menstrual cycle interval between 21 and 35 days as women with regular monthly menstrual cycles are likely to be ovulating. Cervical infertility included 2 women with cervical hostility. Normal ovarian reserve was defined when at least 6 antral follicles were counted per ovary during the early follicular phase.

Folliculogenesis in IUI and IFI

Herein, recruited or pre-ovulatory follicles were defined on the day of ovulation triggering as follicles measuring ≥ 14 mm or ≥ 18 mm mean diameter on ultrasound, respectively.

Due to the duration of subfertility, all patients treated by IUI underwent mild COH as follows: in 20 cycles, gonadotropins (rFSH, Gonal F, Merck Serono, Rome, and/or human menopausal gonadotropin HMG, Meropur, Ferring S.p.A., Milan, and/or rLH, Luveris, Merck Serono, Rome) were started from day 3 of the menstrual cycle; in 5 cycles, anti-estrogens alone (50–100 mg/daily) were started from day 3 of the menstrual cycle and continued for 5 days; in one cycle, a rFSH+GnRh antagonist protocol was used and rFSH was started from day 3 of the menstrual cycle and ganirelix (Orgalutran, Organon, the Netherlands) (0.25 mg daily) was added from day 6 of COH. COH was continued until at least one pre-ovulatory follicle was observed on ultrasound.

IFI was performed as described in previous studies [3–6]. Therefore, moderate COH was applied to most IFI cycles: in 3 cycles gonadotropins (rFSH and/or HMG and/or rLH) were started from day 3 of the menstrual cycle. In order to prevent possible LH premature surge, in 17 more cycles either a ‘long’ GnRh agonist protocol, an rFSH+antagonist, or a modified natural cycle was used. ‘Long’ GnRh agonist cycles were performed by administering buserelin acetate (Suprefact, Aventis Farma) at 0.2 mg bd, starting 1 week before the expected menstrual cycle. Then after bleeding, buserelin acetate was continued at 0.1 mg bd and gonadotropins were added and continued until at least one pre-ovulatory follicle was seen. Protocols for rFSH+GnRh antagonist were performed as described above; the modified natural cycle protocol was performed by administering rFSH (150 units) and ganirelix from day 6 of the cycle. In one more cycle, a natural protocol was used. Folliculogenesis was continued until at least one pre-ovulatory follicle was observed on ultrasound.

In COH cycles, the starting dose of gonadotropins or antiestrogens was established according to the woman’s age and the number of antral follicles, and adjusted according to the number of recruited follicles and endometrial thickness (monitored by transvaginal ultrasonography); estradiol levels were monitored in some cases in order to determine the risk of OHSS at the time of ovulation induction. The possible increase in LH and progesterone was not routinely assessed as cut-off values defining premature luteinization and the decision for cycle cancellation are lacking. Following hormonal and ultrasound check, ovulation was triggered by 10,000 units of human chorionic gonadotropin (hCG, Gonasi, A.M.S.A. S.R.L., Rome) when at least one pre-ovulatory follicle was observed on ultrasound.

IUI or IFI procedures

34.5 hours after hCG injection, semen processing was performed as described [7]; briefly, seminal fluid was treated by density gradient centrifugation (PureCeption, SAGE BioPharma, Bedminster, NJ) and at the end of the separation, all motile spermatozoa were resuspended in 350 μ L of saline. IUI or IFI was performed 36 h after hCG injection. After processing, all recovered motile spermatozoa were inseminated into the uterine cavity to perform IUI, while they were all injected into a single pre-ovulatory follicle or divided into equal parts and injected into 2–4 pre-ovulatory follicles to perform IFI. The number of available spermatozoa, pre-ovulatory follicles, and the woman's age determined the number of pre-ovulatory follicles injected in order to minimize the risk of multiple pregnancy. Most IFI treatments were carried out under local or no anesthesia, a minority under sedation depending on the woman's choice. After careful cleaning of the vagina with sterile saline, pre-ovulatory follicles were injected slowly using a 30 cm 17 gauge oocyte retrieval needle (Cook Ireland Ltd. Limerick). Ultrasound control confirmed that the follicles were not ruptured at the end of the injection. Follicle volume remained unchanged and no fluid was observed in the cul-de-sac.

Luteal phase support

Luteal phase support was added exclusively to cycles in which prevention of premature LH surge was applied [1], beginning from the day of insemination or injection, by using vaginal micronized progesterone at 800 mg/day (Progeffik, Effik Italia S.p.A., Milan, Italy).

Assessment of pregnancy

16 days following IUI or IFI, beta-hCG was assayed in patient serum. In the women who tested repeatedly positive, an ultrasound control was undertaken after 4 weeks to confirm the presence of a fetal heart beat. Biochemical pregnancies (including clinical) were defined as positive (≥ 25 mU/mL) beta-hCG. Clinical pregnancy was defined as an ultrasound-visible fetal heart beat regardless of the site of pregnancy.

Statistics

Statistical comparison was performed by t-test to analyze continuous variables and differences between groups were assessed by two tailed Fisher's exact test for categorical data. The significance level was 0.05. Statistical analyses were performed by using the GraphPad Software.

Results

Couples had suffered from primary ($n=30$) or secondary ($n=8$) subfertility for a mean of 3.4 years (95%CI 2.5 to 4.2). Women were 36.3 (95%CI 35.0 to 37.7) years old, had regular spontaneous menstrual cycles (except 5 displaying oligomenorrhea), a normal body mass index, and a normal ovarian reserve. Bilateral tubal patency was demonstrated in 37 patients, and unilateral patency in one more.

Table 1 summarizes the characteristics of the couples who were treated by IUI or IFI: patients in the two groups were well balanced with regard to female age [36.0 (95 % CI 33.9 to 38.2) vs 36.6 (95 % CI 34.8 to 38.5); $p=0.68$], primary subfertility (15/20 vs 15/18, 75 % versus 83 %; $p=0.69$), duration [3.0 (95 % CI 2.1 to 3.9) vs 3.7 (95 % CI 2.2 to 5.3); $p=0.39$] and cause of subfertility (male factor 10/20 vs 5/18, 50 % vs 28 %; $p=0.19$; unexplained 5/20 vs 9/18, 25 % vs 50 %; $p=0.18$; other factors including mono-lateral tubal occlusion, anovulation, endometriosis, cervical, combined: 7/20 vs 8/18, 35 % vs 44 %; $p=0.75$).

Table 2 summarizes the characteristics of the cycles administered to induce folliculogenesis. As compared to women in the IUI group, women in the IFI group received significantly less COH cycles in which no LH surge suppression was used (3/21 vs 20/26, 14 % vs 77 %; $p=0.0002$), more 'long' GnRh agonist cycles (10/21 vs 0/26, 48 % vs 0 %; $p=0.0001$), more GnRh antagonist cycles (6/21 vs 1/26, 29 % versus 4 %; $p=0.03$), more luteal phase supports (17/21 vs 1/26, 81 % vs 4 %; $p=0.0001$), and were administered more units of gonadotropins [1491 (95 % CI 1025 to 1957) vs 802 (95 % CI 678 to 926); $p=0.009$]. As expected, in the IFI as compared to the IUI group, more follicles were recruited [5.9 (95 % CI 4.2 to 7.5) vs. 2.6 (95 % CI 2.2 to 3.1); $p=0.0001$]; however, the number of

Table 1 Characteristics of the couples

Characteristic	Couples who underwent IUI ($n=20$)	Couples who underwent IFI ($n=18$)
Female age-yr (95 % CI)	36.0 (33.9 to 38.2)	36.6 (34.8 to 38.5)
Primary subfertility-no.(%)	15 (75)	15 (83)
Duration of subfertility-yr (95 % CI)	3.0 (2.1 to 3.9)	3.7 (2.2 to 5.3)
Cause of subfertility-no. (%)		
Mild male factor	10 (50)	5 (28)
Unexplained	5 (25)	9 (50)
Anovulation	3 (15)	2 (11)
Endometriosis	2 (10)	2 (11)
Mono-lateral tubal occlusion	0 (0)	1 (5)
Cervical	1 (5)	1 (5)
Combined	1 (5)	2 (11)

Table 2 Characteristics of the cycles

Characteristic	IUI cycles (<i>n</i> =26)	IFI cycles (<i>n</i> =21)
Stimulation protocol-no. (%)		
COH w/o LH premature surge prevention	20 (77)	3 (14)*
Anti-estrogens	5 (19)	0 (0)
Long GnRh agonist	0 (0)	10 (48)**
rFSH+Gn Rh antagonist	1 (4)	6 (29)***
Natural	0 (0)	1 (5)
Modified natural	0 (0)	1 (5)
Total dose of gonadotropins-units [mean (95 % CI)]	802 (678 to 926)	1491 (1025 to 1957)****
Total dose of antiestrogens-mg [mean (95 % CI)]	360 (88 to 632)	–
Recruited follicles ($\geq$ mm 14 sized)-total no. (95 % CI)	2.6 (2.2 to 3.1)	5.9 (4.2 to 7.5)**
Pre-ovulatory follicles ($\geq$ mm 18 sized)-total no. (95 % CI)	2.6 (2.2 to 3.1)	2.5 (2.1 to 2.9)
Luteal phase support (progesterone)-no.(%)	1 (4)	17 (81)****

* $p=0.0002$, ** $p=0.0001$,
 *** $p=0.03$, **** $p=0.009$

pre-ovulatory follicles at the time of ovulation triggering was similar [2.5 (95 % CI 2.1 to 2.9) vs. 2.6 (95 % CI 2.2 to 3.1); $p=0.53$].

Table 3 summarizes the characteristics of seminal fluid at the time of IUI or IFI and before processing: the two groups did not differ significantly with respect to volume of ejaculate, sperm concentration, motility and normal sperm morphology.

Table 4 summarizes the clinical outcomes following IUI and IFI: the rate of biochemical pregnancy/cycle was 38 % for IFI and 11 % for IUI ($p=0.04$) and the rate of clinical pregnancy/cycle was 29 % for IFI and 11 % for IUI ($p=0.26$). The two groups did not differ with respect to either the cause of infertility (not shown) or the rate of twin gestations and miscarriage. Neither high order multiple gestations nor ectopic ovarian pregnancies were observed; however, 2 ectopic tubal pregnancies occurred in the IFI group, as opposed to none in the IUI group, a difference not statistically significant ($p=0.19$).

Table 5 shows the characteristics of IFI patients and cycles according to the pregnancy outcome: the two groups

did not differ with respect to infertility type and cause, number of recruited or injected follicles, total number of motile spermatozoa injected and normal sperm morphology. Patients who achieved pregnancy were significantly younger than those who failed [34.2 (95 % CI 33.0 to 35.3) vs 37.8 (95 % CI 35.6 to 40.1); $p=0.02$]. Interestingly, more motile spermatozoa were injected/follicle in the patients who achieved pregnancy compared with those who failed [6.9 (95 % CI 3.5 to 10.2) vs 3.1 (95 % CI 0.7 to 5.6); $p=0.02$]. We therefore hypothesized that, apart from the woman's age, also the dose of motile spermatozoa injected/follicle might have been a determinant of pregnancy outcome. We retrospectively observed that all clinical pregnancies ensued in the patients in whom 2.68–6.65 million of motile spermatozoa had been injected/follicle; therefore, IFI cycles were divided into 2 groups (Table 6): group A included 11 patients who were injected with either <2.68 millions ($n=8$) or >6.65 million ($n=3$) motile spermatozoa/follicle, while group B included 10 patients who received 2.68–6.65 million spermatozoa/follicle. Group A and B did not differ with respect to woman's age [36.8 (CI 33.7 to 39.9) vs 35.9 (CI

Table 3 Characteristics of the seminal fluid

Characteristic	Patients who underwent IUI (<i>n</i> =26)	Patients who underwent IFI (<i>n</i> =21)
Volume of the ejaculate-mL	3.5 (2.1–4.8)	3.7 (2.7–4.7)
Sperm concentration-millions/mL	34.0 (23.2–44.7)	47.1 (29.9–64.3)
Sperm motility (PR ^a +NP ^b)-%	41.2 (34.3–48.1)	41.2 (33.8–48.7)
Normal sperm morphology-%	5.9 (5.1–6.7)	5.8 (4.7–6.9)

Values are means (95 % CI)

^a PR progressive

^b NP not progressive

Table 4 Pregnancy outcomes

Characteristic	IUI cycles (<i>n</i> =26)	IFI cycles (<i>n</i> =21)
Biochemical	3 (11)	8 (38)*
Clinical	3 (11)	6 (29)
Twin	0 (0)	1 (5)
High order multiple	0 (0)	0 (0)
Miscarriage	1 (4)	0 (0)
Ectopic (tubal)	0 (0)	2 (9)
Ectopic (ovarian)	0 (0)	0 (0)

Values are no. (%)

* $p=0.04$

Table 5 Characteristics of the patients and cycles in the IFI group according to the pregnancy outcome

Characteristic	Pregnant (<i>n</i> =8)	Non pregnant (<i>n</i> =13)
Woman's age-yr (95 % CI)	34.2 (33.0 to 35.3)	37.8 (35.6 to 40.1)*
Primary infertility-no. (%)	8 (100)	10 (77)
Cause of infertility-no. (%)		
Mild male factor	0 (0)	5 (39)
Unexplained	5 (62)	4 (30)
Other factors ^a	5 (62)	4 (31)
Recruited follicles -no. (95 % CI)	7.6 (5.53 to 9.72)	4.8 (2.49 to 7.05)
Injected follicles-no. (95 % CI)	2.5 (1.87 to 3.13)	2.5 (1.88 to 3.05)
Motile spermatozoa injected-total no. (millions) (95 % CI)	17.9 (6.4 to 29.3)	8.8 (2.7 to 15.0)
Motile spermatozoa injected/follicle -no. (millions) (95 % CI)	6.9 (3.5 to 10.2)	3.1 (0.7 to 5.6)*
Normal sperm morphology-% (95 % CI)	6.7 (4.9 to 8.6)	5.2 (3.7 to 6.7)

**p*=0.02^aIncluded mono-lateral tubal occlusion, anovulation, minimal or mild endometriosis, cervical, more than one cause of subfertility

34.3 to 37.5); *p*=0.57], rate of primary infertility (8/11 vs 10/10, 73 % vs 100 %; *p*=0.21), number of recruited follicles [4.7 (95 % CI 2.7 to 6.8) vs 7.1 (95 % CI 4.4 to 9.8); *p*=0.13] or number of injected follicles [2.4 (95 % CI 1.9 to 2.8) vs 2.6 (95 % CI 1.8 to 3.4); *p*=0.55]. As expected, in group A, in which 8/11 patients were injected with <2.68 million motile spermatozoa/follicle and whose partners displayed worse seminal parameters before processing, sperm morphology was worse as compared with group B. By definition, no clinical pregnancy ensued in group A while 6 clinical pregnancies were observed in group B (*p*=0.04), resulting in 60 % rate/cycle (Fig. 1), of which 2 were ectopic tubal and both patients underwent salpingectomy. In group A we also observed two biochemical pregnancies compared to none in group B (Fig. 1); both ensued in patients who were injected with >6.65 million spermatozoa/ follicle.

Discussion

Although IFI was described several years ago as a new ART technique in which fertilization occurs within the female body [3–6], to date there have been no data to compare IFI

with IUI. This study suggests that, under the conditions described herein, IFI was more effective than IUI at achieving pregnancy and resulted in an interesting clinical pregnancy rate of 60 % per cycle when injecting 2.68–6.65 million spermatozoa/follicle. This is the first study to compare IFI with IUI and may contribute to a revisitation of an ART described many years ago. The clinical use of a new ART technique mainly depends on its reliability, practicability, and costs. IFI appears as a potentially interesting ART, confirming previous suggestions [7]; similarly, IFI has a good practicability as it can be carried under local anesthesia and does not need expensive laboratory equipments. Practicability may be further increased by using natural or minimally stimulated cycles to induce folliculogenesis instead of moderate COH. Costs of IFI are higher compared with IUI as, unlike IUI, IFI is a surgical procedure.

Several biological reasons may explain our results. First of all, in IFI, the Fallopian tubes do not provide the entire mechanism for oocyte fertilization, including sperm ascension towards the fertilization site. Also, the occurrence of fertilization in follicles is biologically plausible; in fact, human follicular fluid is an efficient capacitating agent acting on sperm survival, acrosome reaction, sperm motility

Table 6 Characteristic of IFI cycles according to the number and type of motile spermatozoa injected/ follicle

Characteristic	Group A (<i>n</i> =11)	Group B (<i>n</i> =10)
Woman's age-yr (95 % CI)	36.8 (33.7 to 39.9)	35.9 (34.3 to 37.5)
Clinical pregnancy-no. (%)	0 (0)	6 (60)*
Primary infertility-no. (%)	8 (73)	10 (100)
Recruited follicles-no. (95 % CI)	4.7 (2.7 to 6.8)	7.1 (4.4 to 9.8)
Injected follicles-no. (95 % CI)	2.4 (1.9 to 2.8)	2.6 (1.8 to 3.4)
Motile spermatozoa injected/millions per follicle	< 2.68 (<i>n</i> =8) or >6.65 (<i>n</i> =3)	2.68–6.65
Normal sperm morphology- % (95 % CI)	4.4 (3.1 to 5.6)	7.4 (6.0 to 8.8)**

p*=0.04; *p*=0.019

Fig. 1 Shows the pregnancy outcomes according to the number of motile spermatozoa injected/follicle

hyperactivation, spermatozoa-zona pellucida binding, and sperm-oocyte fusion [10, 11]. Moreover, the follicular fluid plays a pivotal role on motility and chemotaxis of spermatozoa [12, 13] and seems to promote capacitation and acrosome reaction [10].

We hypothesize that in IFI the number of motile spermatozoa injected/follicle may be a determinant of outcome; actually, no pregnancy ensued when fewer than 2.68 million spermatozoa were injected /follicle. According to our hypotheses, Nuojua-Huttunen *et al.* [3] obtained very poor results (2 % pregnancy rate/IFI cycle) by injecting 0.2–0.8 million motile spermatozoa/follicle, a much lower number than our previous [7] and other more successful studies [4–6]. In this study, all clinical pregnancies ensued when 2.68–6.65 million motile spermatozoa were injected/follicle, at the remarkable rate of 60 %/cycle. Interestingly, above 6.65 million motile spermatozoa injected/follicle, only biochemical pregnancies were observed. This could be due to the occurrence of polyspermy and the generation of abnormal embryos observed in mammals when a high concentration of capacitated spermatozoa was used at the site of fertilization [14].

This study is retrospective and therefore lacks randomization; however, a truly free of biases randomized clinical trial may not be correctly designed unless a similar degree of invasiveness is provided to both IFI and IUI patients. As an example, IUI patients should undergo puncture of all follicles before insemination in order to exclude a possible positive effect of the puncture itself on the pregnancy rate, and this may result unethical for IUI patients. Rather, patients were allowed to choose their preferred treatment. Also, the study population was relatively small. Although the aim of this study was only to compare the clinical efficacy of standard IUI with IFI as has been described in

previous studies, it may be still useful to infer possible relevant mechanisms to explain the observed differences. The higher rate of pregnancy observed in patients treated by IFI compared with IUI might merely be due to the higher number of recruited follicles in the IFI group and therefore a higher rate of tubal fertilization occurring after the dehiscence of all recruited follicles; this hypotheses, although not completely ruled out, may be considered less solid by the marginal number of multiple gestations in the IFI group. The observed differences among the two groups might be still due to the different cycles used to induce folliculogenesis; however, the higher dose of gonadotropins in the IFI compared to the IUI group is in favour of IFI, whose efficacy at achieving pregnancy may have been lowered by possible impairment of embryo implantation due to a highly stimulated endometrium. This point can be elucidated in the future by exclusively enrolling patients in whom a single follicle is recruited in a natural cycle, a strategy which has not been used in this study, as it was aimed at comparing a standard IUI technique with IFI as they have been described in the literature [3–6]. A study design in which only natural cycles are used might also exclude a possible impaired potential for embryo implantation and minimize the endometrial factor as a possible further cause for the occurrence of biochemical pregnancies observed in the IFI group.

A matter of concern in this study is the occurrence of 2 ectopic tubal pregnancies in the IFI group, compared with none in the IUI group. This may certainly be due to the small population analyzed; however, alternative hypotheses must be taken into consideration. Among them, the higher effectiveness of IFI in generating embryos and therefore a higher “embryo trafficking” rate within possibly dysfunctional Fallopian tubes has to be taken into account; also, an inhibition of the Fallopian tube epithelial ciliary beat frequency might have occurred, possibly caused by progesterone administered as a luteal phase support to most patients undergoing IFI and to one single patient treated by IUI [15, 16]. Interestingly, in the largest IFI series published so far, no progesterone was needed because LH spontaneous surge was not suppressed and the rate of ectopic pregnancy was found very low at 0.9 % per cycle [6].

In conclusion, under the conditions described herein, we observed more pregnancies in IFI than in IUI. Larger studies to confirm or negate an increased risk of ectopic pregnancy and determine its etiology in IFI are warranted. Further larger and well designed clinical trials may confirm the results reported herein.

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Conflict of interest There are no vested interests to be declared.

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