Feasibility and results of a modified intrafollicular insemination technique for treating primary infertility

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Introduction

Intrafollicular spermatozoa insemination (IFI) was proposed several years ago in one case report and two case series reporting 13–28% intrauterine pregnancies achieved per cycle [1–3]. Despite those encouraging results, IFI was considered ineffective on the basis of a single case series reporting a very poor pregnancy rate per cycle of 2% [4].

Few technical details have been provided in the literature, but a thorough analysis reveals some possible pitfalls in the design, patient selection and technical interventions that explain the largely variable results [1–4].

The purpose of this pilot study is to describe the feasibility and results of a modified approach to IFI.

Materials and methods

Nine consecutive couples were suffering 3.3 years (95% CI 1.9 to 4.6) primary infertility classified as male ($n=2$, one oligozoospermic and one teratozoospermic), unexplained

($n=3$), anovulatory ($n=2$) and due to endometrioma ($n=1$) or mixed factors (cervical hostility and endometrioma) ($n=1$). Female and male partners were 35.2 (95% CI 32.9 to 37.5) and 39.8 (95% CI 34.7 to 44.8) years old respectively. All patients had regular 28-day menses (except two anovulatory displaying oligomenorrhea), showed a normal body mass index and displayed at least six antral follicles per ovary. Seminal fluid was evaluated according to the criteria of the World Health Organization (WHO) 2010. Tubal patency was demonstrated by hysterosalpingography. Patients' karyotypes were requested for all patients in accordance with the national guidelines [5] and found normal in all cases. 5 out of the 9 patients previously underwent at least one uneventful intrauterine insemination (IUI). Before the procedure, the patients signed an informed consent form and agreed to a possible publication of their anonymous data. The Institutional Review Board approval was obtained from the local committee and the study respects the Helsinki Declaration of 1975 regarding human experimentation.

Controlled ovarian hyperstimulation (COH) was administered to the patients by recombinant FSH (rFSH, Gonal F, Merck Serono, Rome) ($n=7$), human menopausal gonadotropin (HMG, Meropur, Ferring S.p.A., Milan) ($n=1$) or both drugs ($n=1$). Patients underwent a long GnRh agonist protocol by using buserelin acetate (Suprefact, Aventis Farma) at 0.2 mg bidaily, then 0.1 mg daily after bleeding, a short GnRh antagonist protocol using ganirelix (Orgalutran, Organon, the Netherlands) at 0.25 mg daily starting from day 6 of COH, or a protocol in which no LH surge suppression was used (Table 1). Following hormonal and ultrasound check, ovulation was triggered by 10,000 units

Capsule Nine couples suffering primary infertility underwent intrafollicular spermatozoa insemination. Four pregnancies ensued: two normal babies were delivered and one pregnancy is presently ongoing.

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Table 1 Shows main demographic characteristics of the study population and principal findings

Patient #	Female age (years)	Infertility causes	Previous IUI cycles (n)	COH cycle	FSH/HMG total dosage (IU)	Pre-ovulatory follicles	Injected follicles (n)	Injected motile spermatozoa/ follicle (million)	Beta hCG (%)	Clinical pregnancies
1	31	Ovulatory	1	Short Antagonist	1,125	4	3	10.45	0	
2	36	Cervical hostility and endometrioma	1	Long Agonist	3,000	4	3	2.68	1	1
3	34	Ovulatory	0	No LH suppression	675	4	2	6.65	1	1
4	41	Unexplained	0	No LH suppression	700	10	2	1.23	0	
5	32	Unexplained	4	Long Agonist	3,525	10	3	11.04	1	
6	38	Male	0	Long Agonist	1,500	5	3	0.70	0	
7	35	Unexplained	1	Long Agonist	1,762	10	3	3.87	1	1
8	36	Endometrioma	1	Short Antagonist	900	8	3	1.73	0	
9	34	Male	0	Long Agonist	1,425	8	2	0.52	0	
Mean/total number	35.2				1,404	7.0	2.7	4.3	4 (44.4%)	3 (33.3%)
95% CI	32.9 to 37.5				639 to 2,170	4.9 to 9.1	2.3 to 3.0	1.2 to 7.5		

of human chorionic gonadotropin (hCG, Gonasi, A.M.S.A. S.R.L., Rome) when at least 3 follicles measured 18 mm mean diameter.

After 34.5 h from hCG injection, semen processing was started 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. IFI was performed after 36 h from hCG injection under sedation in 7 out of 9 patients; after careful cleansing of the vaginal fornices with sterile saline, $\geq$ 18 mm sized follicles were injected slowly with an equal volume of the spermatozoa suspension by using a 30 cm 17 gauge oocyte retrieval needle (Cook Ireland Ltd. Limerick). Ultrasound control confirmed that all follicles injected were unruptured at the end of the insemination; after injection, follicles volume remained unchanged and no fluid was observed in the cul-de-sac. Luteal phase support was offered beginning from the day of IFI by using vaginal micronized progesterone at 800 mg/day (EFFIK ITALIA S. p.A., Milan).

Results

On the day of IFI the initial sperm volume, concentration, total motility and normal morphology were 4.1 mL (95% CI 1.8 to 6.3), 36.5 million/mL (95% CI 18.0 to 54.9), 46.6% (95% CI 39.5 to 53.6) and 5.8% (95% CI 3.8 to 7.7) respectively. After sperm processing, all available motile spermatozoa were injected into 2 to 3 follicles in order to inseminate the maximal number into each pre-ovulatory follicle. A mean of 4.3 (95% CI 1.2 to 7.5) million motile spermatozoa/follicle were injected into a mean of 2.7 (95% CI 2.3 to 3.0) pre-ovulatory follicles (Table 1).

16 days following injection, serum beta-hCG tested positive in 4 patients (44.4% biochemical pregnancy rate per cycle), doubled in 3 patients and tested negative in one patient 2 days later. Six weeks after the procedure, an ultrasound control confirmed the presence of 3 intrauterine singleton pregnancies (33.3% clinical pregnancy rate per cycle) (Table 1), with a visible embryonic heart beat and a gestational age corresponding to 8 weeks as assessed by crown rump length. Pregnancies ensued in couples with the following main infertility factors: mixed (cervical hostility and endometrioma) ($n=1$), anovulatory ($n=1$) and unexplained ($n=2$) (Table 1). Although statistical analysis is inappropriate in this study, couples who got pregnant were similar to those who did not for female ages, gonadotropin dosage, number of pre-ovulatory and injected follicles, while previous uneventful IUI treatments were more represented and the mean number of motile spermatozoa injected per follicle was higher in those who got pregnant compared to those who did not (6 versus 2.9 million)

(Table 1); also, spermatozoa with normal morphology did not differ among the two groups (6% versus 5%, not shown). Of the 3 clinical pregnancies, two continued uneventfully until the vaginal delivery of normal babies weighing 3,200 and 2,990 g at 38 and 41 weeks gestational age (GA) respectively. The third pregnancy is ongoing. Neither early nor late side effects or complications were recorded.

Discussion

In this small series describing 9 consecutive couples, the IFI technique was modified achieving interesting biochemical and clinical pregnancy rates compared with the two largest case series reported so far [3, 4]. Our hypothesis was that, by modifying patient selection and technical interventions, IFI may prove beneficial to a subgroup of infertile couples. Several biological reasons may explain our encouraging 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 hyperactivation, spermatozoa-zona pellucida binding and sperm-oocyte fusion [6, 7]. Moreover the follicular fluid plays a pivotal role on motility and chemotaxis of spermatozoa [8, 9] and seems to promote capacitation and acrosome reaction [6].

Nuojua-Huttunen et al. [4] reported a very poor 2% pregnancy rate per cycle in a series of 50 couples. Factors that may explain at least in part those poor results are the duration of infertility (5.8 ± 3.5 years vs 3.3 ± 1.8 in our study) and the percentage of patients who previously underwent an uneventful embryo transfer (29% vs no patient in our study), possibly implying different patient recruitment criteria. However, those factors may be compensated for by the difference in female partners ages (32.1 vs 35.2 years in our study) and the rate of primary infertility (58% vs 100% in our study). Indications for treatment are similar, apart from the rate of male infertility (38% vs 22% in our study), a difference quite surely due to the different WHO classification used to evaluate semen samples with overestimation of male factor in the case series of Nuojua-Huttunen et al. [4]. Three other issues which may explain the observed differences must be addressed: 1) the number of pre-ovulatory follicles at the time of IFI; 2) the number of follicles injected; 3) the number of motile spermatozoa injected per follicle. Taking into consideration the hypotheses that, in IFI, fertilization may actually occur also or only in the tubes after the dehiscence of the follicles, the different number of pre-ovulatory follicles (3.2 vs 7 in our study) might explain

differences in pregnancy rates, a possibility not completely ruled out by the absence of multifetal pregnancies in our study. The study of Nuojua-Huttunen et al. [4] does not report clearly how many follicles were injected and the number can only be inferred by the reported number of pre-ovulatory follicles. If this is the case, the two studies do not differ significantly (3.2 ± 1.4 vs 2.7 ± 0.5 in our study); also, in our study, patients who got pregnant are similar to those who did not for the number of pre-ovulatory follicles. Importantly, Nuojua-Huttunen et al. [4] have injected 0.2–0.8 millions motile spermatozoa per follicle, a much lower number than that injected in our and other more successful studies [2, 3]; also, in our study, patients who got pregnant received a higher number of motile spermatozoa per follicle, compared to those who did not. Within the limitations of the present study, we suggest that the number of motile spermatozoa injected per pre-ovulatory follicle may be determinant for success.

The main limitation of this pilot study is the small number of patients treated, that prevents any firm conclusion about the efficacy of our protocol. The European IVF Monitoring Program reported a pregnancy rate per IUI cycle around 12% in 2007 [10]. To demonstrate a difference as significant as a 21% increase in pregnancy rate for IFI versus IUI, a total of 38 cycles in each group must be included to reject equivalence between IFI and the standard IUI, considering neither IUI or IFI detrimental (one sided) and with a power of 80% [11].

In conclusion, IFI as described herein appears a feasible approach to treat some couples with long lasting primary infertility. In the future, a better patient selection and a well designed randomized controlled trial to compare IUI and IFI seem appropriate.

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

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