Three In Vivo Fertilization Techniques In Unexplained Infertility And *Male* Factor Infertility: Direct Intraperitoneal Insemination, Fallopian Tube Sperm Perfusion And intrauterine Insemination

İZAH EDİLEMEYEN İNFERTİLİTE VE ERKEK FAKTÖRÜ OLGULARINDA ÜÇ FARKLI İN VİVO FERTİLİZASYON TEKNİĞİ: DİREKT İNTRAPERİTONEAL İNSEMİNASYON, FALLOP TÜPLERİNE SPERM PERFÜZYONU VE İN TRAU TERİN İNSEMİNASYON

Semra KAHRAMAN*, Rita KOLVİK, Lars Wb HALİSKEN****

* SSK Maternity Hospital, Department of Obstetrics and Gynecology, Infertility and Endocrinology Unit, Ankara, TURKEY
** Haugesund Hospital, Department of Obstetrics and Gynecology, IVF and Other Assisted Reproductive Techniques Unit, Haugesund, NORWAY

SUMMARY

Objective: Evaluation of the results of three in-vivo fertilization techniques in unexplained infertility and male factor infertility: Direct intraperitoneal insemination. Fallopian tube sperm pedusion and intrauterine insemination.

***Institution:** Haugesund Hospital, Department of Obstetrics and Gynecology, IVF and Other Reproductive Techniques Unit, Haugesund, Norway*

***Material and Method:** In 73 couples with unexplained infertility and male factor infertility, three asisted reproductive techniques were applied to a total of 89 gonadotropin stimulated cycles. Three different in-vivo fertilization techniques were used [Intrauterine insemination (IUI), Fallopian tube sperm perfusion (FSP) and direct intraperitoneal insemination (DIP!)].*

***Findings:** In the unexplained infertility group six pregnancies were obtained in a total of fourteen treatment cycles with DIP! (42.8%). In the same group one pregnancy was obtained in the IUI group in a total of twelve treatment cycles (22.2%). FSP was employed yielding four pregnancies from a total of sixteen treatment cycles (21.6%).*
In the male factor group with 31 treatment cycles, two pregnancies were achieved with the IUI method (6.5%). In the same group two pregnancies method (6.5%). In the same group two the pregnancies were obtained in a total of nine treatment cycles in the DIPI group (22.2%). The FSP technique was employed yielding two pregnancies from a total of seven treatment cycles.

Results: When the IUI method was employed, the pregnancy rate was quite low in both the unexplained and male factor infertility groups. The clinical pregnancy rate from the DIPI method was higher than that obtained from the IUI method in the unexplained infertility group (P0.05).

Key Words: In vivo fertilization tehniques unexplained infertility male factor infertility DIPI, FSP, IUI

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ÖZET

***Amaç:** İzah edilemeyen infertilité ve erkek faktörü olgularında üç farklı in-vivo fertilizasyon tekniğinin sonuçlarının değerlendirilmesi.*

***Çalışmanın yapıldığı yer:** Haugesund Hospital, Department of Obstetrics and Gynecology, IVF and Other Asisted Reproductive Techniques Unit, Haugesund, Norway*

***Materyal ve Metod:** İzah edilemeyen infertilité ve erkek faktörü olan 73 infertil çifte toplam 89 gonadotropin stimülasyon siklusunda intrauterin inseminasyon (IUI), direkt intraperitoneal inseminasyon (DIPI) ve Fallop tüplerine sperm perfüzyonu (FSP) olmak üzere üç farklı in-vivo fertilizasyon tekniği uygulanarak sonuçlar kıyaslandı.*

***Bulgular:** izah edilemeyen infertilité grubunda DIPI ile 14 tedavi siklusunda 6 gebelik elde edildi (%42.8). Aynı grupta FSP ile 16 siklusta 4 gebelik elde edildi (%21.6). IUI grubunda ise 12 tedavi siklusunda sadece 1 gebelik elde edildi (%8.3) ve spontan abortus ile sonlandı.*
Male faktör grubunda 19 çifte 31 tedavi siklusunda IUI uygulandı ve sadece 2 gebelik elde edildi (%6.5). DIPI ile 8 çifte 9 tedavi siklusu uygulandı ve 2 gebelik elde edildi (%22.2) FSP uygulaması ile 7 çifte 7 teda vi siklusu uygulanması ile 2 gebelik elde edildi (%28.6).

***Sonuç:** Hem izah edilemeyen infertilité hem de male faktör grubunda IUI yöntemi uygulandığında gebelik oranı oldukça düşük bulundu. DIPI yöntemi uygulandığında özellikle izah edilemeyen infertilité grubunda IUI yöntemine kıyasla daha yüksek gebelik oranları elde edildi (P 0.05).*

Anahtar Kelimeler: in vivo fertilizasyon teknikleri izah edilemeyen infertilité erkek faktörüne bağlı infertilité DIPI, FSP, IUI

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Yazışma Adresi: Semra Kahraman
SSK Ankara Hastanesi Kadın Kadın Hastalıkları ve Doğum Servisi Ankara

Currently, agreement has not been reached as to the precise way to treat couples with unexplained infertility. Superovulation together with (DPI), (FSP) or intrauterine insemination (IUI) have been recommen-

ded as alternative treatments to in vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT) as they are non-invasive, less time-consuming and more cost-effective treatments.

With DIPI, it is suggested that if more oocytes are obtained and more spermatozoa selected, the possibility of conception is increased (1).

FSP is a treatment which combines controlled ovarian hyperstimulation, ovulation induction and intrauterine insemination. It was applied for the first time by Kahn et ai. in 1992 (2).

In unexplained infertility, results obtained with DIPI and FSP techniques have been comparable to those with IVF and GIFT (3). However, the role of IUI in the treatment of unexplained infertility and male subfertility is the subject of some debate.

In our study, the three techniques of IUI, FSP and DIPI were compared in unexplained and male factor infertility groups.

MATERIAL AND METHODS

This study was done in the Assisted Reproductive Techniques Unit of Haugesund Hospital In Norway between August 1991 and June 1992. Seventy-three infertile couples with unexplained infertility or male factor infertility were grouped into IUI, DIPI, and FSP groups. The history of infertility for all couples was limited to three years.

All patients had normal hysterosalpingography, ovulation documented with biphasic basal body temperature recordings, and luteal phase progesterone levels. Prolactin levels were normal in ail patients, and laparoscopy showed tubal patency. The criteria for male subfertility were <15x10 million spermatozoa/ml, <30% progressivity and <40% normal forms.

All women received ovarian hyperstimulation. The standard stimulation protocol was a combination of clomiphene citrate (CC; Pergotime; Serono; Italy) and human menopausal gonadotropin (hMG; Pergonal; Serono; Italy), 100 mg CC was given on the fourth menstrual cycle day for five days, and hMG on the seventh day. The ovarian response was monitored by daily measurements of estradiol (E2) combined with regular vaginal ultrasound examinations. Human chorionic gonadotropin (hCG; Physex; Leo; Denmark) was administered for ovulation induction. The maturation of two to four follicles was considered optimal. A serum E2 level greater than 2.4 nmol/ml, E2 elevation over six days, and a minimum of three follicles with diameters greater than 15 mm were required. 6000 IU hCG was administered by IM injection 32-56 hours after the final hMG injection.

Sperm was prepared by the conventional swim-up technique (7). Split semen samples were used. Semen was washed twice by mixing and centrifuging with culture medium. The medium used for swim-up and insemination was Earle's Balanced Salt Solutions (EBSS; GIBCO Ltd; Paisley; United Kingdom) supplemented with Medicult SSR2 (Medicult A/S, Denmark), 1a serum albumin, pyruvate and penicillin. The washed sperm was analyzed in a Makler Chamber (Sefi Medical Instrument Ltd) and then kept in the incubator until insemination.

In the DIPI patients, insemination was performed 36 to 37 hours later in the dorsal lithotomy position without local anesthetic. Ten mg valium was given rectally for analgesia. For the DIPI procedure, 0.8 ml of the washed sperm sample was aspirated into a tuberculin syringe (Gilette; Sabre; Berkshire; United Kingdom) and injected using a GIFT needle Into the posterior cul-de-sac through the posterior wall of the vagina after aspiration of peritoneal fluid. The ovaries were routinely squeezed lightly after the insemination procedure in order to cause rupture of any unruptured follicles.

FSP was performed with the patient in the trendelenburg position. The vagina and cervix were rinsed with IVF culture medium, and a 5 cc plastic syringe

Table 1. A comparison of age distrubition, infertility period and infertility reasons of the three treatment groups
Tablo 1. Üç tedavi grubunda, yaş dağılımı, infertilité süresi ve infertilité nedenine göre kıyaslama.

n: Couples	IUI (n: 31) Mean	FSP (n: 23) Mean	DIPI (n: 19) Mean
Age (Years)*	31.4	32.3	30.8
Range	26-39	26-41	27-39
Infertility Period*	4.8	6.0	5.8
Range	4-9	3-10	4-8
Unexplained Infertility (n:39)	12	16	11
Male Factor (n: 34)	19	7	8

*: Non-Significant

Table 2. The Average number of both inseminated motile spermatozoa and treatment cycles of couples
Tablo 2. İnsemine edilen motil sperm sayısı ve çiftlerin tedavi sikluslarının ortalaması.

The Avarage No.of Ins.Motile Sperm N: Cycles	IUI n: 43 X+Sx	FSP n: 23 X+Sx	DIPI n: 23 X+Sx
Spermatozoa No.*	4.7+2.11	11.2+2.36	6.3+1.34
Average No.of Treatment Cycles of Couples	1.3	1.0	1.2

•Spermatozoa Numbers *10 (Million)

Table **3.** A comparison of the results of the three treatment groups according to the infertility reasons
Tablo **3.** Infertilité nedenine göre üç tedavi grubunda sonuçların kıyaslanması.

Unexplained (n:39)	IUI (n:12)	DIPI (n:11)	FSP (n:16)
Cycles	12*	14*	16*
No.of Pregnancies	1 (8.3%)	6 (42.8%)	4(21.6%)
No.of Miscarriages	1	2	2
No.of Deliveries		4	2
Male Factor	**IUI (n:19)**	**DIPI (n:8)**	**FSP (n:7)**
Cycles	31	9	7
No.of Pregnancies	2 (6.5%)	2 (22.2%)	2 (28.6%)
No.of Miscarriages			
No.of Deliveries	2	2	2

*p<0.05

was filled with 4 ml of IVF medium and sperm suspension. A Frydmann catheter for embryo transfer was inserted via the cervical canal into the upper part of the uterine cavity. Insemination was performed slowly, at a rate of approximately 1 ml per minute. Two clamps were placed in lateral symmetry on the cervix to prevent reflux, and were maintained in this position for the insemination. The patient then rested in bed for one hour after the procedure.

Statistical analysis: Results were analyzed using Fisher Chi-Square test. A p-value of <0.05 was considered statistically significant.

RESULTS

A comparison of three treatment groups with regard to age distrubition, infertility period and infertility etiology are shown in Table 1.

The average number of both inseminated motile spermatozoa and treatment cycles of couples can be seen in Table 2.

A comparison of the results of the three treatment groups according to the etiology of infertility is shown in Table 3.

The unexplained infertility group comprised 39 couples. Out of 16 FSP treatment cycles of 16 couples, 4 pregnancies were obtained (21.6%). Two of them delivered healthy babies at term, while the other **2** pregnancies terminated in spontaneous abortion. In the same group, 6 pregnancies were obtained in a total of 14 treatment cycles with DIPI (42.8%). From the IUI method, the pregnancy rate was quite low; only one pregnancy resulted from a total of 12 treatment cycles (8.3%). There was a statistically significant difference between the results of DIPI and IUI (p<0.05) in the unexplained infertility group. There was no statistically significant difference between the FSP group as compared to the IUI and DIPI groups.

In the male factor infertility group, two pregnancies were obtained in a total of 9 treatment cycles (22.2%) and were delivered healthily at term. In the same group, 2 pregnancies resulted from a total of 7 treatment cycles (28.6%), also yielding healthy term babies. The pregnancy rate in the IUI group was again low. In this subgroup, consisting of 19 couples with 31 treatment cycles, 2 pregnancies were attained (6.5%) and resulted in 2 healthy deliveries at term. There was no statistically significant difference between the three treatment methods in the male factor infertility group (p>0.05).

DISCUSSION

Until now, variable and conflicting results have been obtained in the treatment of unexplained infertility and male factor infertility (407). In most studies, the combination of ovarian hyperstimulation and IUI in unexplained infertility has resulted in pregnancy rates of 0-12% (6). Thus, other treatment models are being developed such as DIPI, FSH, IVF and GIFT.

The first pregnancy with the DIPI method was achieved in 1985. This less-invasive method has been subsequently appiled by other groups in cases of cervical factor, unexplained and male factor infertility.

In 1986, Forrler et al. reported a 14% pregnancy rate in 56 treatment cycles (8,9). The results described by Curson and Persons were not as encouraging: only one pregnancy was achieved in 10 cycles; Jenkins et al. also achieved only one pregnancy in 33 cycles (9-11). Better results were reported by Studd et al, although the series was small (12). Lesec et al reported a pregnancy rate of 7% for male factor infertility (13).

The pregnancy rates in DIPI depend on the quality of ovulation induction and the number of spermatozoa injected. In our study, eight pregnancies were obtained in 23 cycles (34.7% per treatment cycle) in which DIPI was applied. These results are surprisingly good and differed significantly from those obtained in the IUI group. The multiple pregnancy rate was also highest in the DIPI group.

All of the literature on DIPI confirms the importance of seminal properties related to the pregnancy rates. In our study, the number of motile spermatozoa which resulted in conception was as low as 1.9 and 2.1 million and as high as 29x10 million. The highest number of motile spermatozoa resulted in triplets. There are reports of pregnancies after 200.000 and 500.000 motile spermatozoa hava been injected (8,12). It should be considered that in DIPI there are two other mechanisms to increase the fertilization rate obtaining higher numbers of follicles/oocytes and motile spermatozoa. Thus, higher pregnancy rates may also

result. However, the number of embryos and multiple pregnancy rates may be uncontrollable. The figures published for twins are consistently higher in the literature (9,13). The induction of superovulation has significantly raised pregnancy rates for spontaneous cycles with IVF and IUI (14,15). In stimulated cycles with the DIPI treatment, the increased volume of peritoneal fluid is favorable for the survival and capacitation of spermatozoa (16).

Superovulation has two main risks: ovarian hyperstimulation syndrome and multiple pregnancy (16). In our study, a few cases of mild hyperstimulation were observed but required no treatment. Superovulation and DIPI carry the risk of multiple pregnancy, as the number of the eggs to be fertilized cannot be controlled.

The pregnancy rates were 42.8% per treatment cycle in the DIPI group with unexplained infertility and 22.2% with male factor infertility. Thus, DIPI can be employed as an effective and non-traumatic procedure. It is particularly suited for cases of oligoasthenospermia of medium/high severity (17,18).

FSP is simple IUI method. It has recently been developed and applied in Norway. FSP combines controlled ovarian hyperstimulation, ovulation induction and intrauterine insemination of a 4 ml sperm suspension at the time of ovulation. The indications for FSP are the same as for DIPI. The results of the clinical studies of Kahn et al. show that the group with unexplained infertility benefited from the FSP treatment. It was believed that an increased number of gametes at the ovulation site increased the pregnancy rate. In total, the pregnancy rate for this group was 28.6% per treatment cycle in the male factor group. This is comparable with the results obtained in a large controlled multicenter study treating unexplained infertility with GIFT (27%) and IVF (28%) (2).

The disadvantage of the three treatment methods is the inability to confirm fertilization of oocyctes. IVF can be used as a diagnostic procedure to determine fertilization defects (19,20). In their IVF program, Tambo et al. found a high rate of cleavage failure in patients with unexplained infertility (24% per oocyte retrieval). In their studies, 87% of the cycles In which the retrieved oocytes did not fertilize were placed in the group of patients with patent fallopian tubes. It Is believed that IVF may be recommended as the first treatment for patients who have patent fallopian tubes. If fertilization occurs but a pregnancy is not obtained, these patients are subsequently referred for other insemination methods (1).

In our study, although the number of patients was low, the results show that a higher pregnancy rate was obtained in the DIPI and FSP groups than in the IUI group in both unexplained and male factor infertility groups.

The clinical pregnancy rates obtained from DIPI in unexplained infertility were higher than those obtained from the IUI method, and there was a statistically significant difference between these two groups. However, prospective randomized studies in larger groups are needed to confirm the results of the present study.

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