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Impact of Timing on Insemination In Relation to Ovulation on the Cycle Pregnancy Rate of Intrauterine Insemination and Intrauterine Tuboperitoneal Insemination in Unexplained Infertility

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Abstract

Objective:

- To compare the cycle pregnancy rate of intrauterine insemination (IUI) to that of intrauterine tuboperitoneal insemination (IUTPI) in unexplained infertility.
- To assess the effect of timing of insemination in relation to ovulation on the cycle pregnancy rate of IUI and IUTPI.

Design:

Prospective randomized study.

Main outcome measures:

- Cycle pregnancy rate of IUI and IUTPI.
- Cycle pregnancy rate of preovulatory and postovulatory insemination .

Material and Methods: Two groups (A and B), each group included 160 women with unexplained primary infertility. Group A were treated by IUI and group B by IUTPI after mild controlled ovarian stimulation (mCOS) with clomiphene citrate/human menopausal gonadotropin/human chorionic gonadotropin. At the time of insemination the occurrence of ovulation was checked by transvaginal sonography.

Results: After the three treatment cycles 40 patents of group A (25%) and 60 patients of group B (37.50%) had ongoing pregnancies (p=0.033) and the overall cycle pregnancy rate of group A was 9.21% and group B 14.81% (p=0.0324). In group A the cycle pregnancy rate of preovulatory insemination was 7.20% and postovulatory 9.76% (p=0.041). In group B the cycle pregnancy rate of preovulatory insemination was 17.70% and postovulatory 13.0% (p=0.0322). Five out of 40 pregnancies (12.5%) in group A, and 4 out of 60 pregnancies (6.60%) in group B were twins (p=0.0431).

Conclusion: In unexplained primary infertility IUPI had significantly higher cycle pregnancy rate than IUI. Cycle pregnancy rate of IUI was significantly higher with postovulatory than preovulatory insemination. Cycle pregnancy rate of IUTPI was significantly higher with preovulatory than postovulatory insemination.

Keywords: Unexplained infertility; Intrauterine insemination; Intrauterine tuboperitoneal insemination; Mild controlled ovarian stimulation; Preovulatory and postovulatory insemination.

Introduction

Intrauterine insemination (IUI) is considered the first treatment option for unexplained infertility after failure of expectant treatment and before in vitro fertilization [1]. The reported pregnancy rates per cycle usually varied between 8% and 22% [2], but very low (3.28%) pregnancy rates were also reported [3]. The rationale of IUI treatment is to increase the rate of conception in the couple of unexplained infertility by increasing the chance that maximum number of healthy sperm reaches the site of fertilization [3]. It was observed that the number of spermatozoa distribution within the fallopian tubes around ovulation after IUI was low. Only a median of 251 spermatozoa were recorded by flushing the tubes [4] and there was only a 49% chance of peritoneal spermatozoa to be found even when all semen characteristics were normal [5]. Many methods have been tried to improve the outcome of IUI including certain ovarian stimulation with improvement of pregnancy rate [2], perturbation 1 day before IUI [3] but Aboulghar et al. [6] found no significant difference in pregnancy rate with and without hydrotubation before IUI, Fallopian tube sperm perfusion (FSP) [7,8] and intrauterine tuboperitoneal insemination (IUTPI) [9] using 4 ml and 10 ml inseminate

respectively. Four milliliters of inseminate carrying spermatozoa were not sufficient to fill the uterus and Fallopian tubes and did not reach the pouch of Douglas. Ten ml inseminate used for IUTPI filled the uterine cavity and allowed inseminate and spermatozoa to reach the fallopian tubes and enter the peritoneal cavity [9]. Human menopausal gonadotropin (HMG) ovarian stimulation and IUI treatment had a pregnancy rate of 12% per cycle and multiple birth rates averaging 13% [5]. Mildly stimulated (1-3 follicles) cycles might reduce the cost and multiple birth rates but may require more cycles for treatment [6]. The efficacy of IUI/mild controlled ovarian hyperstimulation (mOCS) needs to be confirmed by larger studies [2]. The impact of follicle rupture at the time of insemination on the pregnancy rate in IUI is a debated issue [10]. This factor has not studied in IUTPI. This prospective study was designed to compare the cycle pregnancy rate of IUTPI/mCOS with those of IUI/mOCS and to estimate the association between the timing of insemination with IUI and IUTPI in relation to ovulation (preovulatory or postovulatory) and the cycle pregnancy rate in unexplained infertility.

Material and Methods

The study was carried out between June 2009 and July 2014 at Department of Obstetrics and Gynecology, Tanta University, and a private infertility clinic in Tanta, Egypt. The study was prospective including 320 women with the diagnosis of unexplained primary infertility. They were divided into two groups, A and B, 160 women in each group. The two groups were matched for age, body mass index (BMI) and duration of infertility. They were randomized for treatment by IUI/mCOH (group A) or by IUTPI/mCOS (group B). All patients were complaining of primary infertility for at least 3 years but not more than 6 years. Unexplained infertility was diagnosed after a normal basic fertility evaluation was proved. This evaluation consisted of general and gynecological examinations which were normal. Menstrual pattern was normal, regular cycles of 24-35 days and ovulatory. Spontaneous ovulation was checked by normal midluteal serum progesterone concentration of ≥10ng/ml. Serum prolactin, thyroid hormones, thyroid stimulating hormone and testosterone were normal. If any abnormality detected the case was excluded. Chlamydia detection tests were negative. Normal patent fallopian tubes and normal endometrial cavity were demonstrated by hysterosalpingography and occasionally by laparoscopy because of suspected tubal disease and to assure absence of endometriosis and pelvic adhesions. On day 3 of the menstrual cycle serum follicle stimulating hormone (FSH) was estimated and transvaginal ultrasonography was done to exclude any abnormal uterine findings, to count the antral follicles in the ovaries and to rule out ovarian cysts prior to mCOS. Ovarian reserve was normal as denoted by day 3 FSH <10 mIU/ml and the total antral count, number of antral follicles, 2-8 mm mean diameter in the two ovaries >10 [1]. Age of all participants was ≤ 30 years and their body mass index was <25 kg/m2. The husbands had ≥ 5th percentile of semen parameters according to World Health Organization criteria, 1999 [11] volume 2 ml, concentration 20 million/ml, progressive motility a and b 50%, normal morphology with strict criteria ≥ 4%.The protocol of the study was approved by the committee of Medical Ethics of Tanta University Hospitals. Details of the study were explained to all participants and they signed an informed consent.

Exclusion Criteria

Thyroid and other endocrine disorders, liver and renal diseases, diabetes mellitus, previous pelvic operations or pelvic cervical atresia, cervicitis, endometritis, bilateral tubal obstruction, abnormal menstrual pattern, organic gynecological lesions as leiomyomata, endometriosis, ovarian tumors and cysts and congenital malformations of the genital tract, was administered hormonal treatment in the last 3 months or was submitted to previous assisted conception treatment. All participants accepted 3 treatment cycles if pregnancy was not achieved in the first and second cycles.

Protocol of Mild Ovarian Hyper stimulation

All patients in the two groups underwent the same controlled ovarian hyperstimulation protocol. Clomiphene citrate (CC) 100mg daily for 5 days started from day 3 of the menstrual cycle followed by one ampoule daily of human menopausal gonadotropin (HMG) (75 IU LH + 75IU FSH), menogon (Nile pharmaceutic, Cairo, Egypt) was injected i.m. daily from day 8 of the cycle. The HMG dose was titrated against ovarian response to obtain 1 to 3 follicles of 18 to 20 mm mean diameter as shown by serial transvaginal sonography (TVS). Monitoring started on day 10 of the cycle and repeated every other day until the day of ovulation triggering The ovulatory human chorionic gonadotropin (HCG) {(Pregnyl , Nile pharmaceutic "Organon", Cairo, Egypt), a dose of 10000 IU} was given when mean diameter of the leading follicle reached ≥ 18 mm. Cycles were cancelled when large follicles (mean diameter ≥ 16 mm) were more than 4 in number (to avoid multiple pregnancy) and/or when medium-sized follicles (mean diameter 12-15 mm) were ≥ 10 in number (to avoid hyperstimulation syndrome). Owing to sequential use of CC and HMG, and the variable HMG dose regimen used, rarely HCG administration had to be cancelled. The insemination procedure was scheduled for 34-38 hours after HCG injection (The ESHRE Capri Workshop Group, 2009) [12]. At the time of insemination the occurrence of ovulation was checked by TVS before the procedure. Ovulation was diagnosed by evidence of follicular rupture as shown by the disappearance of the follicle or collapse of the follicle that was reduced in size by ≥ 50% with irregular outline or the follicle filled in with low-level echoes denoting blood and the presence of fluid in Douglas pouch [13].

Randomization

Patients were randomized after administration of HCG

Dark closed envelopes contained the intervention (IUI or IUTPI) were created through the computer by a third party not involved in the allocation process. Randomization was

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performed by picking one envelope for each patient from sequentially numbered envelopes on the day of insemination by a nurse not involved in the study and the patient was informed about the allocated arm.

Semen Processing

For IUI

Semen was collected by masturbation into a sterile jar after 3 days of sexual abstinence. The standard swim-up was used employing Medi-Cult a/s supplemented with human serum albumin (Medi-Cult a/s, Copenhagen, Denmark). The liquefied ejaculate transferred to a sterile labeled 15 ml round-bottomed disposable centrifuge tube (Falcon No 2005) and 4-5 ml of flushing media (Medi-Cult) added to 1-3 ml of liquefied semen. After gentle thorough mixing the sample was centrifuged at 500 xg for 10 min. Then, the supernatant was discarded and the pellet was re-suspended and mixed in 5 ml of fresh media (Medi-Cult) and centrifuged at 500 xg for 10 min. Once again the supernatant was discarded. The pellet was gently layered with media, 1.0 ml and incubated with 5% carbon dioxide, in humidified air mixture for 1 h at 37°C the tube was put in the incubator tilted 45°C. The supernatant was aspirated. The solution was analyzed to evaluate inseminate motile count [14].

For IUTPI

A two-layer gradient technique was used for sperm preparation. One ml of 45%SpermGrad (Vitrolife, Kungsbacka, Sweden)+Ham's F-10 (Gibgo BRL, Life Technologies, Paislet, Scotland) was layered over 1 ml of 90% Sperm Grad, in a 15-ml conical-bottomed centrifuge tube. The undiluted semen was layered over this gradient and the tube was centrifuged for 20 minutes at 250 × g. The supernatant was discarded and the pellet diluted in 7 ml MediCult and recentrifuged for 8 min at 500 × g. The supernatant was discarded and the pellet was diluted in 10 ml MediCult. The solution was analyzed to evaluate inseminate motile count [9]. We routinely practiced the standard swim-up method for IUI. In this study a two-layer gradient technique was used for IUTPI because it was the technique used by Mamas, the first investigator who used IUTPI, to compare the results of the current study with that of Mamas as he achieved high cycle pregnancy rate (30%). However, there was no significant difference between the insemination motile counts (IMC) obtained from the swim-up and the two-layer gradient techniques.

Intrauterine Insemination (IUI)

After cleaning the cervix and vaginal fornices with cotton swabs soaked with physiological saline, IUI was performed using an intrauterine catheter (Krema Delafontaine, Promimed, and Neuilly-en-Thelle, France) with one ml syringe. To eliminate dead space problem, IUI catheter was first attached to syringe and then inseminate was aspirated. The catheter was gently passed through the cervical canal and the sperm suspension (1.0 ml) expelled into the upper part of the uterine cavity. The women remained supine for 30 min after IUI [7].

Intrauterine Tuboperitoneal Insemination (IUTPI)

Intrauterine tubopeitoneal insemination with 10 ml of inseminate was performed using the double nobe bivalve speculum (Parthenon Medical, Athens, Greece) which was specially designed for clamping and sealing the cervix The cervix and vaginal fornices were cleaned with a cotton swab after being immersed in saline. Insemination was carried out slowly in 2-3 min, using an 18 mm insemination catheter (Wallace-Smiths, Hythe, Kent, UK) while simultaneously checking the intrauterine pressure with a Schultze manometer (Schultze, Tuttlingen, Germany). The mean pressure ranged from 80-140 mmHg. As the pressure, gradually built up in the uterine cavity, allowed inseminate to overcome the tubal ostia and, by flushing the fallopian tubes, reached the pouch of Douglas. Intraperitoneal insemination was thus achieved. The fall of intrauterine pressure to 10-30 mmHg and the decrease of the syringe piston resistance denoted the passage of inseminate to the fallopian tubes. Before removing the catheter aspiration by the syringe was tried. If more than one ml was aspirated, it was re-injected and catheter was not removed until no inseminate was aspirated. The women remained supine for 30 min after IUTPI [9].

Luteal Support

After insemination the luteal phase was supported in all participants by daily vaginal supplementation of 400 mg micronized progesterone until day of HCG test performed 14 days after insemination. The end point of the treatment cycle was either a negative pregnancy test or a positive test (serum b-HCG) confirmed by clinical evidence of pregnancy in the form of intrauterine gestational sac and fetal heart pulsation by TVS 2 weeks after positive pregnancy test [8].

Statistical Analysis

Values were recorded as mean ± SD using Microsoft excels version 4. Statistical analysis was performed by Chi-square and t-student tests using SPSS software. Statistical significance was considered when P value was <0.05 [15].

Results

The clinical characteristics of the two groups including IMC were not significantly different Table 1. In the first treatment cycle in group A, 17 out of 160 women were pregnant (10.62%) and in group B, 28 out of 160 women were pregnant (17.50%). The difference was statistically significant (p=0.0431). In the second treatment cycle, in group A, 12 out of 143 women were pregnant (8.39%) and in group B, 18 out of 132 women were pregnant (13.63%). The difference was statistically significant (p=0.0442). In the third treatment cycle, in group A, 11 out of 131 women were pregnant (8.39%) and in group B, 14 out of 114 women were pregnant (12.28%). The difference was statistically significant (p=0.0432). The pregnancy rate per couple in 160 women of group A (IUI) in the three treatment

cycles was 40 pregnancies (25.0%) and in group B (IUTPI) was 60 pregnancies in 160 women (37.50%). The difference was statistically significant (p=0.0332). In group A 160 patients had been submitted to 434 treatment cycles with cycle pregnancy rate of 9.21%. In group B, 160 patients had been submitted to 405 treatment cycles with cycle pregnancy rate of 14.81%. The difference between the two groups was statistically significant (p=0.0324). There were 5 twin gestations out of 40 pregnancies (12.50%) in group A and 4 twin gestations out of 60 pregnancies (6.66%) in group B. The difference was statistically significant (p=0.0431), Table 2. In group A preovulation insemination was done in 92 out 434 cycles (22.11%) and 7 were pregnant, (cycle pregnancy rate of 7.20%) and postovulation insemination in 338 (77.88%) and 33 were pregnant, (cycle pregnancy rate of 9.76%). The difference was statistically significant (p=0.041). In group B, preovulation insemination was done in 72 out of 405 cycles (17.77%) and 18 were pregnant, (cycle pregnancy rate of 25.0%) and postovulation insemination in 323 (79.75%) and 42 were pregnant (cycle pregnancy rate of 13.0%). The difference was statistically significant (p=0.0322), Table 3.

Table 1: Clinical characteristics of women, with primary infertility treated by IUI/mCOS and IUTPI/mCOS; BMI=Body Mass Index; IUI=Intrauterine Insemination; IUTPI=Intrauterine Tuboperitoneal Insemination; IMC=Insemination Motile Count; mCOS=Mild Ovarian Hyperstimulation.

Characteristics	Group A (IUI)	Group B (IUTPI)	P value
Number of patients	160	160	
Age	26.61±2.3	26.80±1.2	0.0821
BMI (kg/m 2)	22.40±2.1	22.12±1.1	0.436
Duration of infertility (years)	4.94±1.1	4.88±1.3	0.242
IMC (million)	16.23±4.31	16.74±2.23	0.361

Table 2: Cycle pregnancy rate of unexplained infertility treated with 3 cycles of IUI/mCOS (group A) and 3 cycles of IUTPI/mCOS (group B); IUI=Intrauterine insemination; IUTPI=Intrauterine tuboperitpneal insemination; COS=Mild Ovarian Hyperstimulation.

	IUI		IUTPI		P value
	N	%	N	%	
1-Treatment cycles: First treatment cycle	17/160	10.62	28/160	17.50	0.0413
Second treatment cycle	12/143	8.39	13/132	13.63	0.0442
Third treatment cycle	11/131	8.39	14/114	12.28	0.0432
Total pregnancy rate/ cycle	40/434	9.21	60/405	14.81	0.0332
Pregnancy Rate/couple	40/160	25.0	60/160	37.5	0.0334
2-Twin pregnancies	5/40	12.50	4/60	6.66	0.0431

Table 3: Cycle pregnancy rate with preovulatory and postovulatory insemination in IUI and IUTPI; *CPR was significantly higher with postovulation than preovulation insemination (P=0.041), **CPR was significantly higher with preovulation than postovulation insemination (p=0.0322); IUI=Intrauterine Insemination; IUTPI=Intrauterine Tuboperitoneal Insemination; CPR=Cycle Pregnancy Rate

Insemination cycles	Preovulatory insemination		Postovulatory insemination	
	N (%)	CPR N (%)	N (%)	CPR N (%)
IUI (n=434)	96 (22.11)	7 (7.29)	338 (77.88)	33 (9.76)*
IUTPI (n=405)	72 (17.77)	18 (25.0)	323 (79.75)	42 (13.0)**

Discussion

The present study included two groups of women with unexplained infertility. Group A was treated by IUI/mCOS and group B by IUTPI/mCOS. The two groups were matched for age, BMI, menstrual pattern and type and duration of infertility. Table1 the overall cycle pregnancy rate of 3 treatment cycles of IUI/mCOS, group A, was 9.21% Table 2, similar to that of many previous studies, 10.0% [16], 10.1% [17] and 12% [5]. The present study and the 3 studies mentioned above had no significant differences in any of the confounding variables affecting the cycle pregnancy rate, age of participants, type and duration of infertility, stimulation protocol, number of follicle per cycle, endometrial thickness, semen parameters and timing and number of treatment cycles. In the present study, the overall cycle pregnancy rate of 3 treatment cycles in group B (IUTPI/mCOS) was 14.81% Table 2. Mamas [9] reported, under the same conditions, a rate of 29.4%, nearly double the rate of our study. Thangwijitra et al. [18] achieved one pregnancy in 16 stimulated cycles of IUTPI for 16 cases of unexplained infertility, a cycle pregnancy rate of 6.30%. There were no significant differences in any of the confounding variables among the three studies except the number of treatment cycles in the latter study. We followed exactly the same technique described by Mamas [9]. Thangwijitra et al. [18] did not use DNB speculum but a size 8 F pediatric Foley's catheter with a balloon inflated in the endometrial cavity to block the internal os of the cervix to prevent reflux of the inseminate during infusion. They believed that this catheter and its balloon may inflict some damage to the endometrium inducing an endometrial reaction with leucocytic infiltration that may hinder ovum implantation and may predispose to preclinical or early miscarriage. Thangwijitra et al. [18] reported presence of blood on the catheter after insemination in 13 out of 16 cases submitted to IUTPI. This may

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explain the low cycle pregnancy rate (6.30%) in their study compared to the rate reported by Mamas [9]. Recently, a study of Mamas et al. [19] indicated that sperm pooling combined with IUTPI may be a useful technique in the treatment of mild male infertility.

To increase the pregnancy rate of insemination, direct intraperitoneal insemination (DIPI) was performed by injection of washed sperm into the peritoneal cavity by puncturing the vaginal cul-de-sac at the time of ovulation in hopes of achieving fertilization and pregnancy. Turhan et al. [20] used DIPI for the treatment of 254 cycles of unexplained infertility together with mild ovarian stimulation. The cycle pregnancy rate in this study was 18.5%. Direct intraperitoneal insemination by vaginal wall puncture, carries a risk of intraperitoneal infection .and pelvic adhesions [20].

Intrauterine tuboperitoneal insemination has been practiced, since 2002, by Mammas et al. [21]. Before its clinical application many studies showed that spermatozoa and oocytes, fertilized or unfertilized deposited in pouch of Douglas would enter the fallopian tubes. Coulam et al. [22] presented the technique of peritoneal ovum-sperm transfer as an option for treating couples with unexplained infertility. In 1989 they reported the first successful pregnancy after transferring sperm and oocytes into the pouch of Douglas through the posterior vaginal fornix. In 1991 they reported the results of a prospective study of this procedure. Twelve women with unexplained infertility underwent 23 cycles of peritoneal ovum-sperm transfer with 20% live births. Ovaries were stimulated by HMG.

Initial experience with a modification of the follicle aspiration, sperm injection, and assisted rupture (FASIAR) was practiced. A transvaginal ultrasound–guided needle was used to puncture mature follicle in the ovary. The follicular fluid was aspirated with oocyte into a syringe that also held the semen. This mixture was then immediately injected back into the follicle. Because the total injected volume was much greater than the volume of the original follicle, the fluid containing sperm and oocyte was noted to flow out of the follicle into the pouch of Douglas. They reported 21% live birth per cycle [23].

Wanggren [24] using gamma camera imaging and autoradiography found evidence of uptake of radio-labeled particles in the fallopian tubes after deposition into the pouch of Douglas at the time of laparoscopy. The particles were also detected in the cervical mucus. Uptake of particles in the fallopian tubes suggested a retrograde transport mechanism. Muscular contractions of the fallopian tubes are regulated by prostaglandins and progesterone; their receptors are expressed in the human fallopian tube [24]. It is believed that in mammals, including human, there is sperm chemotaxis to oocytes. First spermatozoa may be chemotactically guided to the oocyte-cumulus complex by the gradient of progesterone secreted from cumulus cells. Next, while within cumulus matrix, spermatozoa may sense the more potent attractant that is secreted from the oocyte and be chemotactically guided to the oocyte and penetrate it [25]. Branchi et al. [26] discovered interacting proteins on the surface of the sperm and oocytes. The protein on the surface of the sperm was called Izumo after marriage shrine and on the surface of oocyte is called Jumo after the Roman Goddess of fertility and marriage. The authors found that pairing of Jumo and Izumo is necessary for fertilization. The Izumo-Jumo pairing is the first known essential interaction for sperm-egg recognition in any organism. They found that after the initial fertilization step there is sudden loss of Jumo from the surface of oocyte, becoming virtually undetectable by sperm. This may explain why the oocyte once fertilized by the first sperm cell loses its ability to recognize further sperm [26]. Matson et al. [27] showed that oocytes may still be retrieved from the pouch of Douglas, despite follicle dispersal; these oocytes can be fertilized, and the embryos derived from ovulated oocytes recovered from the pouch of Douglas may generate an ongoing pregnancy following in vitro fertilization and embryo transfer [27]. The typical coronal section of the uterus, tubes and ovaries that is frequently used to illustrate the anatomy of the female pelvic organs shows that relative positions as they would appear stretched out on a dissecting board rather than in vivo. This anatomical representation facilitates the theory that the ovulated oocyte is picked by a sweeping motion of the fimbriae of the ipsilateral tube over the surface of the ovary [28]. However, in vivo the fimbrial ends of the tubes are located posterior to the uterus within the pouch of Douglas beneath the ovaries. Picturing the anatomy in this way makes it easier to understand how oocytes can be extruded into the Douglas pouch within the follicular and peritoneal fluid where they may be picked by either Fallopian tube. Ovum pick-up from the cul-de-sac probably occurs reasonably frequently and is unlikely to have a causative role in the pathogenesis of ectopic pregnancy through ovum transmigration [29]. The above studies [22-29] denote that sperm and/or oocytes, even if fertilized, deposited in pouch of Douglas may enter the fallopian tube and pass to the uterine cavity and produce normal pregnancy. This may explain the mechanism of successful pregnancy with IUTPI even if insemination was done after ovulation and oocyte was flushed from tubal lumen to the pouch of Douglas. The volume of 10 ml of inseminate used in IUTPI, similar to the action of hysterosalpingography, may have a mechanical effect (increased intrauterine pressure), that may remove by force the tubal plug which has been noted in some tubes in unexplained infertility. This plug appears as amorphous matter on histological examination of these tubes and this mechanical effect also opens loose adhesions that may be present around the fimbriae [30]. Peritoneal and follicular fluids around the time of ovulation in spontaneous and gonadotropin stimulated cycles were obtained from the pouch of Douglas during diagnostic laparoscopy and gamete intrafallopian transfer procedures. The effects of these fluids on the percentage of motile spermatozoa were studied by in vitro objective motility assessment and compared to control media. Overall, these fluids from spontaneous and stimulated cycles sustained and increased sperm viability and motility [31]. Peritoneal and follicular fluids from hormonally stimulated cycles sustained sperm motility better than those from spontaneous cycles. These fluids influenced progressive motility of the sperm positively while sustaining the number of motile spermatozoa [21] The results of Lopez-Gatius and Yaniz [32] showed that following intraperitoneal insemination, there was passive sperm transport from the peritoneal cavity to the genital tract close to the time of ovulation, and suggested higher sperm retention in

the genital tract when live as opposed to dead spermatozoa. Chen et al. [33] studied the effect of peritoneal fluid collected in early follicular phase of the menstrual cycle on sperm motility parameters in women with unexplained infertility. They compared the effect of normal peritoneal fluid and human tubal fluid with 10% fetal bovine serum, as culture media, on sperm after swim-up preparation. The mixture of sperm and peritoneal fluid or human tubal fluid with 10% fetal bovine serum (control) was analyzed at 0, 1, 3, 6 and 24 hours of co-incubation using the computer-assisted analysis (CASA) system. At the end of the 24 hours incubation, supravital staining of sperm was done to check the viability of sperm in each group. Only time has a significant ($p<0.001$) effect on sperm motion parameters. At 6 h, sperm velocity (mean curvilinear and mean straight line velocity) was significantly greater than that of control. At 24 h, the peritoneal fluid group maintained 50% of initial sperm viability, compared with 13% of the initial viability in the tubal fluid with 10% fetal bovine serum ($p<0.001$).

These studies [30-33] proved that the fertilizability of sperm and oocytes is upgraded in Douglas pouch by peritoneal and follicular fluids. On the other hand other studies [34,35] evaluated the clinical efficacy of IUI, FSP and DPI by randomized study. Their results showed that the three techniques of insemination had similar efficacy on the achievement of clinical pregnancy in couples affected by long standing infertility. The number of patients in the previous two studies was small. Mamas [9] performed IUTPI 34-36 hours and Thangwijitra et al. [18] 36-40 hours after HCG injection. The two investigators did not define the timing of insemination in relation to ovulation. Yaniz et al. [36] found that verification of sperm deposition in the proximity of the ovaries improved fertilization rate and the optimal time for intraperitoneal insemination appeared to be just prior to ovulation. The comparison for follicular rupture versus non-rupture at IUI showed significantly higher cycle pregnancy rate with follicular rupture (12.6% versus 6.0%) in unexplained infertility [17]. This is in agreement with the results of the present study as the cycle pregnancy rate of IUI was significantly ($p=0.041$) higher in postovulatory than preovulatory insemination (9.76% versus 7.29%). Moreover, the study of Kucuk [10] suggested that monitoring of follicular rupture prior to IUI provides pregnancy rate similar to normal fecundity. According to the results of his study he suggested that IUI should be withheld until follicular rupture is detected. In the majority of published studies, the insemination is done 32-36 h following HCG administration [12]. It is believed that IUI at 32-38 h post-HCG would produce the best results [37]. The scientific basis for 32-38 h timing is derived from the ultrasonographic and hormonal studies that have shown the occurrence of follicle rupture by day 2 post-hCG in 81% of stimulated cycles [38]. As shown in this study Table 3 the incidence of follicular rupture shown by TVS at the time of IUI was 77.88% which agrees with the above studies and lends credence to choosing the timing of IUI at 36 ± 2 h post-HCG. Rupture of follicle before IUI grantees oocyte availability. It can be rightly argued that the difference in cycle pregnancy rate when ovulation is present and when it is absent at the time of IUI can be explained at least partially by the luteinized unruptured follicle syndrome which may occur in 20% of stimulated cycles by clomiphene citrate/HMG/HCG [39].

In the present study cycle pregnancy rate was significantly (0.0322) higher in preovulatory than postovulatory IUTPI (25.0% versus 13.0%), Table 3. On insemination just before ovulation, sperm were found in the lumen of the tubes and the pouch of Douglas. After ovulation the oocyte was captured to the ampullaary part of the tube and was fertilized by sperm found in the tube and pouch of Douglas as they were chemotactically guided to the tubal lumen after ovulation [26]. On postovulatory IUTPI with 10 ml inseminate the oocyte may be flushed from the tubal lumen to pouch of Douglas where it may be fertilized and may then enter the tube by retrograde transport [26] or may be captured by tubal fimbriae [30]. Fertilization under these unnatural conditions may have detrimental effects on oocytes or zygotes. This may suggest that the best time for IUTPI is just prior to ovulation.

Conclusion

Intrauterine tuboperitoneal insemination had significantly higher cycle pregnancy rate than IUI for treatment of unexplained infertility. Cycle pregnancy rate of IUI was significantly higher with postovulatory than preovulatory insemination. Cycle pregnancy rate of IUTPI was significantly higher with preovulatory than postovulatory insemination.

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