DIRECT INTRAPERITONEAL INSEMINATION AND DIRECT INTRAFOLLICULAR INSEMINATION

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SUMMARY

The present study was made to explore if direct intraperitoneal insemination (DIPI) or direct intrafollicular inseminatiion (DIFI) represent viable option in selected cases of refractory infertility. Out of 12 DIPI treatment cycles in 10 subjects 1 conceived and in 15 women who had 19 DIFI treatment cycles 2 reported with pregnancy. Earlier, all these couples had at least six unsuccessful intra uterine insemination (IUI) treatment cycles. The technique of in vitro sperm capacitation and insemination by echo guided probe is described in detail. Our preliminary clinical experience is reported herewith.

INTRODUCTION

The pioneering work of Stepto and Edwards in reporting the worlds first baby following in-vitro fertilization (IVF) in 1978, heralded a new era of high technology. Newer artificial reproductive techiques (ART) derived from IVF protocol, such as gamete intra fallopian transfer (GIFT), zygote intra fallopian transfer (ZIFT) and micro-insemination have changed the concept of infertility management. Simultaneously, the older method of insemination is improved upon by newer ART. Controlled ovarian hyper-stimulation (COH), ovulation induction, sperm washing in-vitro sperm capacitation and IUI is a standard line of treatment in many centers. Widely variable pregnancy rates ranging from 7-30% have been reported (Dodson and Honey 1991, Banker 1993, Mitra and Chakravoty 1994). Yet majority of subfertile couples with unknown etiology, cervical hostility and poor sperm

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quality fail to conceive. IUI like any other artificial procreation leads to mounting mental stress and every unsucessful attempt adds on tremendous frustration. This study was undertaken to explore if a new approach like DIPI and DIFI represent the alternative viable options before extra corporal fertilization or GIFT are attempted in such a situation.

MATERIALS AND METHODS

A series of 25 subjects were included in this study, for a total 31 treatment cycles. In DIPI 10 couples had total 12 treatment cycles and 15 patients of DIFI were given 19 treatment cycles. Distribution of the cases are seen in Table I.

Table I
Distribution of 25 Cases

	DIPI	DIFI
Number of patients	10	15
Number of treatment cycles	12	19
Average duration of infertility	5 years	5.5 years
Range of duration of infertility	3-15 years	3-13 years
Average age of patients	29 years	30 years
Average IUI cycle offered	8	7
Controlled ovarian hyperstimulation		
A.CC & hCG cycle	10 (1 conceived)	12 (1 conceived)
B.CC 7 hMG & hCG	2 (none conceived)	7 (1 conceived)

The couple are selected for the procedure only after detailed clinical and diagnostic hystero-laparoscopic examination. Proper counselling was done, emphasizing the procedure, possible complications and obtaining an informed consent. All these subjects have at least one healthy patent tube and optimum hormone response. Earlier, all of them had undergone a minimum of six IUI treatment cycles but failed to conceive.

Technique of DIPI: The procedure involves two main steps:

Preparation of aliquot of spermatozoa by the standard sperm washing and "swim up" technique (Sher et al., 1984). Next is insemination, where the processed and activated spermatozoa are deposited in to the cul-de-sac or inside the preovulatory follicular antrum.

Sperm preparation: A fresh semen sample is collected by masturbation in a sterile disposable plastic petri dish with all aseptic precautions, allowed to liquefy for 30 minutes. About 0.5-0.75 ml of liquefied semen sample is diluted in 2.5-3.5 ml Ham's F-10 tissue culture media prepared in our laboratory. The sample is spun at 1000 rpm for five minutes and the supernatant fluid is gently decanted to dispense with the suspended seminal plasma. The pellet containing spermatozoa is washed again by centrifuging in about

2 ml culture media. The final sperm pellet is overland by fresh 0.5 cc culture media enriched with 10% heat inactivated fetal cord blood serum and left for a short incubation at room temperature. The vigorously motile spermatozoa swim up near the surface. A drop of supernantant fluid is examined under microscope. All these women had controlled ovarian hyperstimulation (COH) with clomiphene citrate (CC) 100 mg daily starting from day 3 to day 7 of the cycle alone or together with hMG 75 iu daily from day 7-9 of the cycle. The ovarian response is studied from day 10 of the cycle with transvaginal 5.0 MHz probe and by daily measurement of the diameter of the follicles, endometrial thickness and echo free mucus filled cervical canal. Inj. hCG 5000 iu is administered when the leading follicle reaches a diameter of 18 mm. After 36 hours of hCG injection folliculometry is done more frequently till the signs of ovulation are evident like sudden collapse of the follicle size, fluid in POD and altered echogenic charcter of endometrium.

Insemination: In majority of the cases an appreciable amount of fluid as echo free areas, are evident in the cul-de-sac or around the adnexa for twelve to eighteen hours after ovulation. The fluid accumulates after the rupture of the follicle and extrusion of oocyte. Such fluid collection helps as a guide to deposit the inseminate in the cul-de-sac. The fluid in small pockets are collected in to a larger pool by coalescence maneuvering the trans vaginal probe head carefully. The layers of the cul-de-sac septum is identified. A special 20G needle is inserted and with the help of biopsy guide the needle tip is steered on the video monitor till the same enters the fluid pocket. If the needle placement is accurate, air bubbles can be seen and the fluid can be aspirated. The processed actively motile sperm cells (approx. 10,00000) suspended in about 1.0 ml Hams F-10 culture media is then injected into the pool of peritoneal fluid.

Technique of DIFI : The same procedure is adopted as in DIPI.

After 34 to 36 hours of hCG administration each preovulatory follicle is injected with processed sperm suspended in 0.2 cc of culture media containing approximately 200,000 actively motile spermatozoa. We use same 20 gauge needle and observe that the follicular size continues to remain unchanged for several hours after the puncture; this indicates that there is no immediate outflow of follicular fluid. We use local infiltration of 1% lignocaine. However, few cases are performed under sedation only. No vaginal antiseptic solution is used because of potential toxicity to gametes.

Three patients reported with amenorrhoea and positive urinary hCG. Vaginal USG confirmed itra uterine pregnancy. The first case was of DIFI performed on 16th March 1993, on day 13 of a CC induced cycle in a dominant follicle of only 16 mm diameter. She had history of infertility for 13 years with her husband's mean sperm count of less than 10 million per ml. Earlier she had undergone nine unsuccessful IUI treatment cycles.

Ovarian stimulation : All patients of DIPI and DIFI had COH 22 cycles with CC alone, and 9 with CC and hMG together.

The goal of COH is to stimulate follicles

of uniform size, such that each mature follicle would produce approximately 300 pg/ml of estradiol. This condition ensures optimum follicular maturity. However, in the absence of any supporting hormone lab in our area we had to depend exclusively on USG findings. Besides measuring the follicular size we look for the evidence of cumulus oophorus, contour duplex, a thin echo free line between hypoechoic granulosa complex and echogenic thecal compartment. When the endometrial thickness measures 8 mm or more displaying good stromal edema as evident by hypoechoic area between echogenic central and two peripheral lines, we interpret that circulatory estradiol is optimum and good endometrial response has been achieved. In 4 cycles, the ovulation was not evident even 72 hours after hCG administration. Our attempt to rupture the follicles by bimanual compression failed in 2 subjects. In another 2 subjects the needle tip could not be placed into the ill defined fluid pocket after ovulation. Therefore, we subsequently ventured to perform DIFI in subsequent cycles. We had no major complications but 5 patients complained of mild to moderate abdominal pain. Apparently no vascular or intestinal perforation, though might have occurred inadvertently, produced any complications. We encountered no case of pelvic inflammation or febrile conditions in our present series.

DISCUSSION

DIFI and DIPI are new artificial reproductive procedures. Melnick & Ruhnikor et al (1992) observed excellent results of 32 pregnancies (42%) after 12 cycles of DIFI programs in a total of 77 infertile couples.

Lucena et al (1991) first reported four pregnancies in 14 cases of DIFI. Zbella et al (1992) reported a case of successful pregnancy following DIFI procudure when 3 successive IVF& ET treatment cycles failed in a couple of male factor infertility.

The IVF technique aims at union of gametes in culture media invitro. This occurs in a more physiological environment of oviduct in GIFI. Workers who advocate GIFI claim that biochemically complex tubal fluid contains factor(s) which ensure optimum conditioning of the gametes, offers physiological environment and facilitates fertilization. Does the preovulatory follicular fluid (FF) have similar potential for the union of the gametes?

Ralt et al (1991) observe that FF not only offers a stable environment but unidentified factor present in the FF attracts the sperm and promotes sperm function. The sperm acrosome reaction is a calcium dependent process (Dukelow and William, 1988). Preincubation of sperm with FF helps to increase the influx of calcium through the sperm membrane; thus enhances the ability of the sperm to penetrate the zona pellucida and facilitate fertilization. (Yee and Cummings 1990). Fakih and Vijayakuman (1990) observed improved pregnancy rate following GIFT when FF was used as gamete transfer media following sperm washing and sperm capacitation with FF.

Our observations with preliminary studies are encouraging in IUI resistant cases of infertility. However, a number

of unknown factors are to be explored before these techniques are advocated for wider use. The first question arises whether the increased number of spermatozoa artificially introduced in the peritoneal cavity or in the intra-follicular space induce the formation of more female antisperm antibodies. Secondly, whether the procedures are associated with increased risk of ectopic pregnancy. Is DIFI superior to DIPI ? Theoretically, the peritoneal fluid dilutes the follicular fluid and the sperm concentration at the site of fertilization in DIPI. However, DIFI has further distinct advantages over DIPI in cases of LUF where bimanual menchanical pressure fails to rupture the preovulatory follicle. DIFI might be practiced for LUF patients who fail to ovulate and are destined to have their cycles cancelled?

Like IVF and GIFT techniques, DIFI & DIPI methods are designed to bypass the uterus and Oviduct to bring about fertilization. Therefore, it is to be further explored whether such manipulation of the sperm directly in the peritoneal cavity or inside the follicles facilitates increased pregnancy rates in subjects where there is immunologically mediated sperm inactivation or where uterus is incapable of sperm capacitation. Theoretically, these procedures are to improve the sperm function in cases of immunological infertility. It is to be further explored whether hyper-stimulation with hMG alone or together with CC is superior to CC induced cycle of COH. In our study we observed 2 pregnancies in 22 CC cycles. One conceived following 9 CC & hMG treatment cycles; all conceived in the first treatment cycle.

CONCLUSION

Artificial insemination has come a long way and shall continue to remain an essetial part of infertility treatment. Both DIFI and DIPI are new ART and have a promising role in the treatment of unexplained and male factor infertility, particularly in IUI failed couples. The procedure is simple, less invasive, less expensive, low risk. These may prove an effective option in IUI failed cases. However, further controlled studies are required to establish the validity of this therapeutic approach.

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