ROLE OF INTRA-UTERINE INSEMINATION AND SPERM PREPARATION TECHNIQUES IN TREATMENT OF UNEXPLAINED INFERTILITY AND MALE INFERTILITY

Surg Cdr SUSHIL KUMAR^{*}, Dr HEMANGI AZGAONKAR⁺,
Surg Cmde RT AWASTHI[#], Surg Lcdr ANUPAM KAPOOR⁺⁺,
Surg Lcdr S SRINIVAS⁺⁺

ABSTRACT

Role of intrauterine insemination (IUI) in infertile couples is evaluated. Patients selected for the study belonged to two categories- Unexplained infertility (34 patients) and Male infertility (30 patients). In all the patients selected for IUI ovarian hyperstimulation was carried out with clomiphene citrate. Two techniques of sperm preparation were used - swim-up technique and mono-percoll gradient separation technique. Pregnancy rates of 15.6% per patient and 7% per cycle respectively were achieved which are comparable with other studies. Mono-percoll sperm separation technique was found more effective in treatment of Male infertility (oligo-asthenospermic semen sample).

MJAFI 2000, 56 : 209-212

KEY WORDS: Infertility; Intra-uterine insemination, Male infertility, Sperm preparation.

Introduction

Artificial insemination of husband's semen (AIH) has been used in clinical medicine for more than 200 years in the treatment of infertile couples. The first documented application of AIH was done in 1770 by John Hunter [1]. A patient with severe hypospadias was advised to collect the semen in a warm syringe and inject the sample in to the vagina. J Marion Sims [2] reported his findings of post coital tests and 55 inseminations in mid-1800s, but artificial insemination became popular only after the introduction of sperm donation.

The increasing use of AIH in unexplained and male infertility is mainly the result of the refinement of techniques for preparation of washed motile spermatozoa as they were used in in-vitro fertilization (IVF) procedures. Washing procedures seem to be necessary to remove prostaglandins, infectious agents and antigenic proteins. These techniques also result in the removal of non motile spermatozoa and round cells. These round cells reduce sperms fertilizing capacity.

Intra uterine insemination (IUI) may have a beneficial effect on the number of functional sperms at the site of fertilization. However the role of AIH remains controversial [3-5]. The population treated by AIH is very heterogeneous and ovulation induction regimen, semen preparation techniques and insemination procedures vary widely. Most of the controversies centre on

the role of IUI in treatment of unexplained infertility and male infertility. The present study summarizes the result of 228 intra-uterine inseminations with husband's semen, performed in 64 patients.

Material & Methods

64 infertile couples were included in this study of which 34 were patients of unexplained infertility and 30 couples belonged to the male factor defect group. The diagnosis of unexplained infertility was made when all the following investigations were within normal limits i.e tests for ovulation, tubal patency, cervical factor, tubal and peritoneal factors and semen analysis. The diagnosis of male infertility was made if the semen quality was below the standard laid down by WHO manual 1992 [6]. Patients having sperm count less than 10 million per ml. were excluded from the study since according to various studies, success of IUI in such cases is very poor [7,8].

Procedure

(A) Induction of Ovulation

After selection of patients all the female partners underwent ovarian hyper-stimulation with clomiphene citrate and human chorionic gonadotrophin (hCG) protocol to achieve higher number of oocyte for fertilization. Clomiphene citrate 50 mg was given orally from 5th to 9th day of cycle. Daily follicular study was done from tenth day onwards. Alongwith follicular study cervical score was maintained. When follicle reached 18 mm and with an optimum Insler score, the patient was given hCG 10,000 IU.IM.

(B) Timing of Intrauterine Insemination

Ovulation usually occurs 36 hours after hCG, but in some patients it may occur earlier. Also to maximize the chances of success it is necessary to have spermatozoa present in uterine cavity and tubes at the time of ovulation. To achieve this we did two

^{*}Classified Specialist, ⁺Post Graduate Student, [#]Consultant, ⁺⁺ Graded Specialist, Department of Obstetrics and Gynaecology, INHS Asvini, Mumbai 400 005.

IUIs in each cycle one 12 hours after hCG injection and second 36 hours after the injection. For the sake of convenience, hCG was given at 8 pm and first IUI was done at 8 am next morning. The second IUI was done at 8 am the day after. In each patient the IUI was planned for two cycles. To minimize the bias IUIs were done alternately with the sample prepared by swim-up and monopercoll technique (alternate cycle).

(c) Sperm preparation :

The sample was collected after three days of abstinence in a wide mouth polypropylene container, the method of collection was by masturbation. In cases of severe oligospermia split ejaculate was collected. The collected samples were then kept in an incubator at 37°C for 20 minutes for liquefaction. After liquefaction samples were examined for various sperm parameters. Makler counting chamber was used in all cases for the counting. After analysis the samples were split into equal aliquots, these aliquots were then processed with swim-up or monopercoll gradient technique.

(i) Swim-up technique

The semen sample was layered with Earls balanced salt solution (Sigma Lab) The mixture was then thoroughly mixed with the help of sterile plastic pipette and then centrifuged for 10 minutes at 1500 rpm. Supernatant was carefully removed from the pellet. It was then layered with 0.7 cc of Earls media and kept in the incubator at 37°C, tilted at an angle of 45 degree with the help of a beaker for 30 minutes. After 30 minutes highly motile spermatozoa are expected to swim-up from the pellet. At this stage 0.5 cc of supernatant containing highly motile spermatozoa was aspirated gently.

(ii) Monopercoll gradient separation technique

1 ml 80% percoll was placed in 10 ml sterile conical tube. It was then carefully layered with 1 ml liquefied semen. The gradient was then gently stirred at semen percoll interface leaving 0.5 ml of the bottom end of the tube undisturbed. It was then centrifuged at 1000 r.p.m. for 30 minutes. Normally all the motile spermatozoa travel through the gradient and reach the bottom by forming a pellet, while the debris remains in the upper layer. Supernatant was then removed by carefully skimming the surface with sterile pipette leaving 1 ml at the bottom of the tube. Then 0.5 ml of Earl's medium was added to allow slight resuspension. The pellet was then carefully transferred to a clean sterile centrifuge tube. It was then washed with Earl's medium and resuspended with 0.5 ml of Earl's media and evaluated.

Both the samples prepared by swim-up technique and monopercoll separation technique were evaluated for -

1) Sperm concentration, percentage motility; 2) motile density (sperm count per ml X % motility X semen volume / 100); 3) percentage morphology (number of normal sperm per hundred sperms counted); 4) percentage recovery of motile spermatozoa (final motile density X 100/Initial motile density).

Observations

In this study a total of 64 couples participated and 228 inseminations were performed. Mean number of inseminations per cycle were 1.6 (Table-1). As shown in Tables 2&3, both in normal semen sample and in oligospermic semen samples monopercoll technique significantly improves the sperm concentration, motile density, percentage morphology and also yields significantly larger number of total motile spermatozoa as compared to swim-up technique. Total ten pregnancies occurred in this study; six pregnancies occurred in unexplained infertility and four in patients

with male factor involvement (Table-4). 7% pregnancy rate per cycle and 15.6% per patient was achieved. Out of total ten pregnancies eight were achieved with monopercoll method of sperm recovery and two with swim-up method (Table-5). Out of these pregnancies seven culminated into nine full term normal babies, two were lost to follow-up after 20 weeks of pregnancy and one had spontaneous abortion. There was no major complication of IUI procedure however minor complications occurred in 21 (9.8%) patients. The complications included mild ovarian hyperstimulation in three patients; pelvic inflammatory disease in two patients; abdominal cramps in eight patients and minor bleeding in eight patients. No patient required hospitalization for the same.

TABLE 1

Demographic data - INHS Asvini AIH Program

Number of couples	64
Age of the patients	
Male	32.2 years
Female	28.1 years
Duration of infertility (months)	44
Number of cycles	142
Number of inseminations	
Primary infertility	160
Secondary infertility	68
Mean no. of insemination per cycle	1.6

TABLE 2

Observations on normal semen samples used in cases of unexplained infertility*

	Initial sample	Monopercoll	Swim-up
Sperm conc x 10 ⁶ /ml	60.68 ± 17.34	35.89 ± 14.30	20.27 ± 8.85
Percentage motility	69.95 ± 9.90	88.25 ± 6.06	85.57 ± 8.31
Motile density (10 ⁶ /ml)	43.85 ± 16.58	32.36 ± 13.71	17.90 ± 8.37
Percentage recovery	NA	71.42 ± 14.78	39.81 ± 10.11
Percentage morphology	61.31 ± 8.26	82.31 ± 6.35	77.32 ± 7.2

*Figures are mean ± SD

TABLE 3

Observations on abnormal semen samples used in cases of male factor infertility*

	Initial sample	Monopercoll	Swim-up
Sperm conc x 10 ⁶ /ml	16.14 ± 06.17	4.96 ± 1.70	2.07 ± 1.26
Percentage motility	43.75 ± 14.87	71.88 ± 12.38	63.86 ± 15.10
Motile density (10 ⁶ /ml)	6.58 ± 2.42	3.52 ± 1.19	1.24 ± 0.67
Percentage recovery	NA	55.58 ± 14.16	19.20 ± 8.16
Percentage morphology	39.98 ± 5.92	63.29 ± 4.67	55.80 ± 6.67

*Figures are mean ± SD

Discussion

In this study we used ovarian stimulation protocol with clomiphene in all our patients. Clomiphene is a cheap drug and is readily available. Two of the most popular techniques, swim-up and percoll gradient

TABLE 4

Pregnancy rates achieved in patients with unexplained infertility and male factor infertility

	No. of patients	No. of cycles included	No. of IUIs done	Pregnancies obtained
Total patients	64	142	228	10
Unexplained infertility	34	74	116	6
Male factor infertility	30	68	112	4

TABLE 5

Number of pregnancies achieved as per method of sperm recovery

	In normal semen sample		Male factor semen sample	
	Monopercoll	Swim-up	Monopercoll	Swim-up
No. of pregnancies	4	2	4	0
	Total - 4+2=6		Total - 4+0=4	

methods were used in our study. Sperm recovery and pregnancy rates obtained by these two methods are compared. Most of the studies [9-11] suggest that percoll technique is superior to swim-up, however all of them used more complicated multilayered percoll gradient technique. Only Shailka in 1995 [12] published the results of monopercoll technique. Our results are comparable. It can be concluded that in both normal semen sample and oligospermic sample, monopercoll density gradient separation technique improves all the parameters i.e. sperm concentration, motility, percentage recovery of motile spermatozoa and recovery of morphologically normal sperms. Higher recovery of total motile spermatozoa by percoll density gradient technique in comparison with swim-up could be due to the sub-population of the spermatozoa which while undergoing centrifugation, generate reactive oxygen

species which damage the cell membrane, this in turn hampers motility. In percoll preparation this sub-population of the spermatozoa are already being separated from highly motile and normal spermatozoa as they pass downwards to the bottom. Hence there is no generation of reactive oxygen species. Most of the workers have obtained higher percentage of morphologically normal spermatozoa after percoll compared to swim-up even in abnormal semen samples. The exact mechanism by which percoll improves the morphology is not known. Electron microscopic study showed that the sperms were devoid of acrosomal membrane and there was complete condensation of chromatin material. These changes were similar to acrosome reaction. Percoll technique selected more number of oval headed sperms, of normal morphology as compared to swim - up.

Pregnancies after IUI procedure

Comparison of our study with work of other authors is a very complicated task; the literature is still very confusing. The population treated by AIH is very heterogeneous and ovulation induction regimens, semen preparation technique and insemination procedures vary widely, especially in cases of unexplained infertility and male infertility (Table-6). Our results of 7% pregnancy rate per cycle and 15.5% per patient are almost similar to other studies using CC(clomiphene)hCG protocol. However higher pregnancy rates were obtained by using CC hMG hCG by others [13]. In our study we used the first protocol only since the later protocol was very expensive and was beyond our financial means. As far as IUI for male factor is concerned results are not consistent. Controlled prospective cross over study by Velde et al [14] comparing IUI with timed coitus showed no ad-

TABLE 6

Pregnancies after IUI-Comparative study

Author	No. of patients	Ovarian stimulation protocol	No. of cycles	Pregnancies (% per cycle)	Pregnancy rate per patient
Ombelet (13)	412	A) CC+hCG	548	55 (10)	36.2%
		B) CC+hMG+hCG	508	90 (17.7)	
		C) GnRH+hMG	39	4 (10.2)	
Pratap (15)	380	D) CC+hCG	Total	14.75%	16%
		E) CC+hMG+hCG	1140	17.28%	
Dmowski (16)	27	-	-	4	15%
Karlstorm (17)	-	CC	-	4%	-
Serhal (18)	-	No ovarian stimulation protocol	-	2.7%	-
Our study	64	CC + hCG	142	10 (7%)	15.6%

vantage of IUI like several authors. We in our study had four pregnancies in patients with oligo-spermia. However all these pregnancies were obtained by using monopercoll density gradient technique and no pregnancies with swim-up technique. In cases of unexplained infertility we obtained six pregnancies out of which four were with monopercoll and only two with swim-up. Higher pregnancy rate with monopercoll separation technique in both the groups is comparable with results of other workers [15,16].

Pregnancy outcome

In our series of patients we achieved ten pregnancies out of which one was spontaneous abortion and out of nine other pregnancies, seven delivered live babies and two were lost in follow up after twenty weeks of pregnancy. There was no case of multiple pregnancy. Ombelet [13] in his series found ectopic pregnancy in 1.3% cases, chemical pregnancies in 9.4% and clinical abortion in 14%. Abortion rate of 10% to 20% has been mentioned by other authors [12,15]. Low abortion rate in our study could be due to use of monopercoll separation technique. However larger studies are needed to draw any inference.

Complications

None of our patients required hospitalization due to any complication arising out of ovarian hyper-stimulation protocol or IUI procedure itself. However there were only three cases (4.5%) of mild ovarian hyper-stimulation in form of ovarian enlargement requiring no specific treatment. Others have reported hyper-stimulation rate of 10% [13]. Higher rate of hyper-stimulation in these studies is mainly due to use of hMG for ovarian hyperstimulation while we used only clomiphene in all our patients.

Pelvic inflammatory disease (PID), another complication of intrauterine catheterization, was found in two patients requiring only OPD treatment. We did not use prophylactic antibiotics in our institute and we agree with others that it will be difficult to prove the effectiveness of prophylactic antibiotics for AIH due to low incidence of salpingitis. Other minor complications were abdominal cramps in eight patients and minor bleeding during insertion of IUI catheter in eight patients. These minor complications did not require any specific treatment and did not interfere with the outcome of IUI.

Acknowledgement

Part of this study was conducted under aegis of AFMRC project No 2129/96. I am thankful to Armed Force Research Committee for sanctioning the project. We extend our gratitude to Prof Sadhana K Desai, Prof and Head of the Dept, Bombay Hospital

for providing technical training.

References

1. Siegler SL. Fertility in women. Philadelphia, JB Lippincott, 1944;403.
2. Sims JM. Uterine Surgery. New York, William Wood & Co., 1883;365.
3. Adamson DG, Subak LL, Boltz NL, McNutly M, Failure of intrauterine insemination in refractory infertility population, Fertil Steril, 1991;56:361-3.
4. Ho PC, So WK, Chan YF, Yeung WS. Intrauterine insemination after ovarian stimulation as a treatment for sub-fertility because of sub-normal semen. Fertil Steril 1992;58:995-9.
5. Yovich JL, Matson PL. The treatment of infertility by high intrauterine insemination of husband's washed spermatozoa. Human Reprod 1998;3:939-43.
6. World Health Organization, Laboratory manual for examination of human semen and sperm cervical mucous interaction, Singapore press. 3rd ed 1992.
7. Arny M, Quagliarello JR. History of artificial - insemination. Reprod Endocrinol 1987;5:1-3.
8. Acosta AA, Kruger TF, Human Spermatozoa in Assisted Reproduction, 2nd ed. The Parthenon Publishing Group 1996;368.
9. Bollendorf A. Comparison of direct swim-up and mini percoll separation procedures. Arch Androl 1994;32:157-62.
10. Jaroudi KA. Percoll semen preparation enhances human oocyte fertilization in male factor infertility as shown by randomized cross over study. Hum Reprod 1993;8:1438-42.
11. le Lannou D. Nuclear maturity and morphology of human spermatozoa selected by percoll density gradient centrifugation or swim-up procedure. J Reprod Fertil 1988;84(2):551-6.
12. Shaikha S. A monopercoll separation technique improves sperm recovery of normal and male factor samples when compared with swim-up technique. Hum Reprod 1995;10(12):3195-7.
13. Ombelet W, Cox A, Janssen M, Vanderput H. Artificial Insemination 2-Using the Husband's sperm. In : Acosta A A, Kruger TF, editors. Human spermatozoa in Assisted reproduction. 2nd ed. London: The Parthenon Publishing group 1996;399-410.
14. Velde ER, Van Kooy RJ, Waterreus JJH. Artificial insemination of husband's sperm. Fertil Steril 1988;49:602.
15. Tord T. Mini percoll, a new method of semen preparation for IVF in severe male factor infertility. Hum. Reprod 1990;5(8):987-9.
16. Kumar Pratap. Results and complications of IUI. Bulletin of the Indian Society for Assisted Reproduction. 1998;1:1.
17. Dmowski WP, Gaynor L, Lawrence M, Rao R. Intra uterine insemination. Fertil Steril 1979;31:58.
18. Karlstorm PO, Berch T, Lundquist O. A prospective trial of artificial insemination versus intercourse in cycles stimulated with hMG or clomiphene citrate, Fertil Steril 1993;59:554.
19. Serhal PF, Katz M, Little V. Unexplained infertility -The value of pergonal superovulation combined with IUI. Fertil Steril 1988;49 : 602.