

GENDER SELECTION BY INTRA- UTERINE INSEMINATION FOR INFERTILE COUPLES

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ABSTRACT

Background: Many centers worldwide used Ericsson method for gender selection. The studies found that sperm with high concentrations of either X or Y bearing sperm could be obtained.

Objective: To examine the effect of modified procedure of Ericsson method on gender selection following intra-uterine insemination (IUI) for infertile couples. **Patients, Materials and Methods:** fifty infertile couples were included in this study. They were randomly divided into two groups Group one , the gender selection protocol was done by discontinuous density gradient(45% and 90%) technique with 7% and 17% albumin concentration technique. Group two simple layer and density gradient techniques were used for gender selection. Then IUI was accomplished and pregnancy test was done following 14 days of insemination to detect the level of *h* CG. The gender of fetus was

obtained by ultrasonography examination from sixteen weeks gestation onward. **Results:** There was a highly significant ($P < 0.01$) improvement in the percentage of sperm motility grade A and B after the activation was recorded after *in vitro* activation by density gradient and albumin concentration techniques compared with simple layer and density gradient techniques. The percentage of morphologically normal sperm following *in vitro* activation was revealed no significant ($P > 0.05$) differences between the two techniques. There was 10 out of fifty women become pregnant (220%). Distribution of live birth babies according to gender using protocol of group two was 6 male babies and the percentage of male sex selection was (66.6%), while only 3 female babies delivered which give a percentage of (33.4%). **Conclusion:** It is concluded that using density gradient and albumin techniques is

more effective than simple layer and density gradient techniques for gender selection when infertile couples seek to have a baby by IUI.

KEYWORDS: Gender selection IUI, density gradient, albumin columns.

INTRODUCTION

Two major types of pre-implantation methods can be used for social gender selection. The Ericsson method, which is used to determine whether enriched sperm samples would result in offspring of a desired gender. This method was first applied in a clinical setting in the 1970s by Dr. Ronald J. Ericsson^[1], and *in vitro* fertilization (IVF) preimplantation genetic diagnosis (PGD) technique in which, the embryos of the desired gender are implanted back in the mother's uterus.^[2] Recent study was carried out in Iraq in which a modified Ericsson method using discontinuous density gradient procedure in different percentage of concentration levels of albumin were used which revealed that the best concentration and motility of sperm obtained by using 7%, and 17% albumin concentration for IUI.^[3]

Although some causes of male infertility are treatable or correctable through surgery, other cases the treatment are unsuccessful or the cause of male infertility is unknown or untreatable, assisted reproductive techniques such as IUI may be suggested in such cases which is commonly used in cases of low sperm count or quality.^[4,5] Consequently, the objective of this study is to examine the effect of modified procedure of Ericsson method on gender selection following IUI for male infertile couples.

PATIENTS, MATERIALS AND METHODS

This study was allocated in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, From October-2013 till Oct -2014 .Fifty infertile couples were included in this study. Men with normozoospermia and mild male factors infertility (those with mild oligozoospermia, mild asthenozoospermia, and those with leukocytospermia) were included.

Methods of gender selection

Using modified Ericsson methods, two different procedures were used in this study. Fifty patient were enrolled in this study and divided into two equal groups (twenty five patients in each). The procedure of sperm activation for each group was.

- 1st group: using *in vitro* sperm activation by discontinuous density gradient centrifugation technique using a concentration of 45%-90% (AllGrad[®], LifeGlobal Company-Belgium) procedure, and then albumin concentration of 7% -17%. The two albumin density gradients 7% and 17% were prepared by the dilution of human albumin 20 % (Biotast Pharma GmbH, Germany) with Hams-F12 medium (Sigma-Aldrich-USA).
- 2nd group: using *in vitro* sperm activation by swim up technique and discontinuous density gradient centrifugation technique using percoll concentration of 45%-90%.

Then 0.5ml of the prepared semen suspension aspirated into 1ml. syringe and attached to endocervical catheter (Genetics, Belgium) after writing the full name of both partners. The preparation ready for insemination.

Preparation for Insemination

1 Ovarian stimulation protocols and ovulation Induction

Protocol of ovulation induction for each woman was as the following: Clomiphene citrate (CC) 50 mg tablet (Aventis, France) alone in dose ranging from (50-150) mg/day from day 2 of the menstrual cycle for 5 days.^[6]

Protocol of Intra- uterine insemination.

The female partner was prepared for insemination process. The loaded IUI catheter was passed through both the external and internal Os using marker guide line for correct placement. Gently inseminate the sperm preparation (0.3-0.5ml sperm suspension) high into the uterine cavity. Gently the speculum was removed and the patient was instructed to spin on the side of ovulating ovary for 30 minutes.^[3]

Follow up of the female partner after insemination.

Luteal phase supplementation was started from the next day after insemination by using progesterone through either oral route using progesterone tablets (Duphaston® 10mg/day, Solvay, Holland) for 2 weeks or Intramuscular route (17- hydroxyl progesterone corporate) 250 IU given twice weekly or vaginal route (Crinone 8%) daily for 2 weeks.^[7] Then a blood sample was obtained from the female to test for beta chain of human chorionic gonadotropin (β- HCG) level, if the result of the test was positive, the patient was undergo follow up and careful antenatal care by an obstetrician with regular visits. An ultra sonography was done to confirm viability of intra uterine pregnancy, fetal growth and development, and at 16 weeks

of gestation ultra sound was confirmed for fetal sex determination.^[7] While women with negative pregnancy test, new cycle of intra uterine insemination done for her.

Statistical analysis

Statistical analysis was performed by using SPSS (statistical package of social science, version 21.0 LED technology, USA) and Microsoft Excel work 2010 for figures. The results were expressed as mean \pm standard deviation (SD). Analysis of variance was applied to differentiate between three groups and when the F value reaches the significant level, least significant test was performed. Chi square was done for non parametric values. The differences between the values were considered statistically significant if the P value was lower than 0.05.^[8]

RESULTS

Table 1: Certain sperm function parameters following the activation *in vitro* using two techniques for gender selection

Certain sperm function parameters		Following <i>in vitro</i> activation techniques			P Value
		Before * activation	DG with Albumin (Group I)	Simple layer and DG (Group II)	
Sperm Concentration (x 10 ⁶ /ml)		53.38 \pm 2.929	32.10 \pm 2.052	26.82 \pm 1.560	P<0.05
Sperm motility (%)	Grade A	5.90 \pm 1.048	30.14 \pm 2.679	24.50 \pm 3.140	P<0.05
	Grade B	35.22 \pm 1.175	45.40 \pm 2.292	54.80 \pm 2.646	P<0.05
	Grade C	26.88 \pm 1.080	15.40 \pm 1.494	13.94 \pm 1.197	NS
	Grade D	32.00 \pm 1.414	9.06 \pm 1.088	7.76 \pm 0.443	NS
Morphologically normal sperm(%)		36.16 \pm 0.976	70.32 \pm 2.055	69.12 \pm 1.624	NS

Values are expressed as mean \pm SD. No. semen samples /group=25 DG :Density gradient ANOVA *P<0.05 significantly different than after activation techniques . NS: non-significant

Certain sperm function parameters following the activation *in vitro* using two techniques for gender selection

The result of present study found a significant decrease in sperm concentration following *in vitro* activation by using DG and Albumin technique (group I) compared to group II technique. The percentage of active sperm motility grade A after using density gradient and albumin concentration techniques was significantly(P<0.05) lower than other activation technique .A significant (P<0.05) improvement in the percentage of active sperm motility grade B, was recorded after using density gradient and albumin concentrations(group I) than

that of and simple layer and density gradient technique(group II) .At the same time the sperm activity grade B in group II was significantly($P<0.05$) increase compared than in group I .There was no significant ($P>0.05$) differences in sperm motility grade C and D when using any of the two techniques in gender selection. The percentage of MNS was shown no significant ($P>0.05$) differences following *in vitro* activation between Group I and Groups II as shown in table (1).

Pregnancy rate following IUI

The pregnancy rate following IUI for gender selection of infertile couples using density gradient and albumin techniques (groupI) was 6 out of 25 patients(24%). The PR in groupII was 4 out of 25 couple ,the percentage of successful pregnancy was 16%.There was a significant ($P<0.05$)increase in PR by using the density gradient and albumin techniques (groupI) compared to swim-up and density gradient techniques(groupII)as shown in(Table-2).The total PR was 20%and the number of male was 66.6% (6 out of 9) and the females were 33.4%(3 out of 9).There was only one aborted baby (1 out 10=10%)

Table(2): Total Pregnancy rate and gender outcome following intra-uterine insemination for gender selection of infertile couples of the two groups

No. patient	pregnancy rate		Total	
	Group I		Group II	
50	6/25	24%	4/25	16%
No. of complete pregnancy		No. of abortion		10/50 20%
GI=5 GII=4	9=90%		GI=1 GII=0	1=10 %
Male		Female		9
GI=4 GII=2	6*=66.6%		GI=1 GII=2	3=33.4%

* $P<0.05$ significant

Chi square

GI=group I GII=group II

DISCUSSION

In vitro preparation techniques

In this work, there was a different modification in the procedure of Ericsson method by using mainly the discontinuous density gradient technique .This technique was used in different IVF centers worldwide because of the best results obtained in the last fraction of sperm with high active motility and morphologically normal sperm percentage ,free from debris, round

cells and immotile spermatozoa.^[3] Therefore in this study, the procedures of preparation by this technique improve both the sperm motility and percentage of morphologically normal sperms (MNS) too.^[9,10]

Using albumin for sperm preparation was enriched the medium with high amount of cyclic adenine monophosphate (cAMP) which improved sperm motility by increasing the number of highly active motile sperms.^[9]

The results of the two procedures used in current work revealed that the techniques in group one namely ; discontinuous density gradient and columns of albumin 7%-17%, were the most suitable method for gender selection of infertile couples . However when the semen samples activated *in vitro* the result yield a reasonable total sperm concentration. This finding may be resulted by the effects of the activation techniques that eliminate the immotile and dead sperms at the same time only active sperms with normal forms will response positively to the techniques used leading to decrease the sperm concentration with increase in active sperm motility and percentage of normal morphology.^[11]

Consequently, the results of active sperm motility were significantly increased in the two groups. This observation can be explained by the positive effect of density gradient technique alone and/or with other techniques(simple layer and albumin columns) leading to increase the percentage of sperm motility and MNS in the two groups.

In this work, out of the fifty couples inseminated, 10 got pregnant (20%), one ends with first trimester abortion while the others got full term pregnancy. It seems that IUI carries essentially the same risks of antenatal and perinatal complications as pregnancies resulting from normal sexual intercourse.^[11]

The total full term pregnancy rate by using , density gradient and albumin techniques was 24% the percentage of male gender was 66.6% and female was 33.4%.This high percentage in male selection contributed to the mixing of two techniques used in gender selection.^[12] This result obtained matches the results obtained by Dr. Ericsson method for sex selection^[1] and Al-Dujaily and Al-Dahan result.^[3]

All the couples of this study are infertile therefore percentage of male sex selection in this study t was 66.6% with percentage of 20% pregnancy rate which in turn interfere with the results of insemination by a sufficient number of male sperms^[13 -15] and may be the sperm

have a genetic problems(i.e DNA fragmentation) resulted from the increase the production of free radicals (i.e. reactive oxygen species) or decrease in antioxidant capacity leading to oxidative stress.^[16]

It is concluded that the best protocol of *in vitro* activation of semen of infertile couples to obtain certain sperm function parameters for gender selection was by using density gradient and albumin columns leading to higher IUI outcome.

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