

## Gender Selection by Ericsson Method in Intrauterine Insemination for Infertile Couples

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### Abstract

- Background** Ericsson method used to determine whether enriched sperm samples would result in offspring of a desired gender. It is used in approximately 50 centers in the United States and in many centers worldwide when scientists and andrologist discovered that sperm samples with high concentrations of either X or Y bearing sperm could be obtained.
- Objective** To examine the effect of Ericsson method on gender selection following intra-uterine insemination for infertile couples.
- Methods** One hundred infertile couples were included in this study. A programmed ovulation induction for women was done. Luteinizing hormone and estradiol hormone level were measured. Ultrasonography was used to detect the number and diameter of follicles and endometrial thickness at menstruation cycle 1 day before human chorionic gonadotropin injection. Semen analysis was done for all husbands and density gradient technique with 7% and 17% albumin concentration was performed for sperm selection *in vitro*. Intra-uterine insemination was accomplished and pregnancy test was done 14 days following insemination to detect the level of human chorionic gonadotropin in blood. The gender of fetus was recorded 4 months following pregnancy by ultrasonography.
- Results** Twenty two (22%) out of one hundred women become pregnant. According to gender, live birth babies distributed into thirteen male babies (76.64%) and only four female babies (23.36%). One pregnant woman delivered twins following intra-uterine insemination.
- Conclusion** It is concluded that Ericson method is a simple and effective technique for gender selection when infertile couples seek to have a baby by intra-uterine insemination.
- Key Words** Gender selection, Infertile couples, Intra-uterine insemination, Ericsson method.

**List of Abbreviation:** E2 = estradiol, FSH = follicular stimulating hormone, hCG = human chorionic gonadotropin, LH = luteinizing hormone, IUI = intrauterine insemination, IVF = in vitro fertilization, PGD = preimplantation genetic diagnosis, TSH = thyroid stimulating hormone.

### Introduction

Over centuries, couples and individuals with or without their partner's knowledge have tried to influence the sex of their babies. Following the ancient Chinese astrological birth chart, couples matched the mother's time of birth to the

month of proposed conception to select a baby's gender. Ancient China still used today <sup>(1)</sup>. The other theory was founded by *Hippocrates, Greek Physician* who believed that male develop on the right side of the *uterus*, and females on the left. Women were instructed to have sex lying on their right side, if they desire a boy <sup>(2)</sup>. The other old theory described by *Leviticus, the Talmud* was instructed the woman to have an *orgasm* before the man to conceive a male, and vice versa for a female baby. Early Greeks

(Anaxagoras -500 to 428 BCE) often tied off the left testicle with twine during intercourse, hoping to produce a son, and the right testicle, a daughter<sup>(3)</sup>. In 20<sup>th</sup> century, scientists prescribed a high-protein diet for women wishing to conceive a boy<sup>(4)</sup>. Sperm sorting utilizes the technique of flow cytometry to analyze and 'sort' spermatozoa. During the early to mid 1980s, Johnson *et al*<sup>(5,6)</sup> was the first to sort viable whole human and animal spermatozoa using a flow cytometer and utilized the sorted motile sperm for artificial insemination.

Recently, 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, was first applied in a clinical setting in the 1970s by Dr. Ronald J. Ericsson<sup>(7)</sup>, and *in vitro* fertilization (IVF)/preimplantation genetic diagnosis (PGD) technique<sup>(8)</sup> in which, the embryos of the desired gender are implanted back in the mother's uterus. Thus, the goal of this work was to examine the effectiveness of Ericsson method to determine the desired gender following IUI of infertile couples.

### **Methods**

This study was carried out in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, through October 2011 till December 2012. One hundred infertile couples were included in this study for gender selection. Couples were carefully managed, through detailed history (IVF indicated cases were excluded) viewing all previous investigations. Men with normozoospermia and mild male infertility factors (those with mild oligozoospermia, mild asthenozoospermia, and those with leucocytospermia) were all included. The spouses with anovulatory causes (except resistant cases of polycystic ovary syndrome and endometriosis), unexplained infertility and hostile post coital test were involved in this study.

### **Female investigation**

The average women age that included in this work was 27.7±3.4 years old (ranged between 21 to 36 years). All the women had full detailed history, and complete physical examination done by the gynecologist. Hormonal analysis was done through menstrual cycle at days 2-3, 11-12 and 21-22 includes serum prolactin hormone, luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH) and estradiol hormone (E<sub>2</sub>). Then at mid luteal phase, progesterone level was measured too. A serial vaginal ultrasonography was carried out to check the ovulation status, size of the uterus, endometrial thickness, size of each ovary, number and size of antral follicles, on day 12-14 of the cycle. The tubal patency was examined by hysterosalpingography and /or diagnostic laparoscopy<sup>(9)</sup>.

### **Male examination**

The husbands were examined by a consultant urologist. Semen sample (100) was obtained via masturbation after an abstinence period of 3-5 days, collected directly into a clean, dry and sterile disposable Petri dish. Each sample was transported to the semen examination laboratory immediately and allowed to liquefy in an incubator at 37 °C. After complete liquefaction, the semen was analyzed by a macroscopic and microscopic examination using the standards of World Health Organization<sup>(10)</sup>.

### ***In vitro* preparation of semen for gender selection**

The semen of infertile men complaining of mild oligoasthenozoospermia was prepared *in vitro* using albumin discontinuous density gradients. 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). The procedure of semen preparation *in vitro* was depending on Ericsson method<sup>(7)</sup> with a modification in using centrifugation at 4000 RPM for 20 minutes. Microscopic examination was done using 10 µl of last fraction from the top of tube and the results of certain sperm characters were reported.

### Intra-uterine Insemination

Hundred spouses who prepared for IUI were involved in this study. About 0.3-0.5 ml of the prepared semen was aspirated into 1 ml syringe and attached to endo-cervical catheter (Gynetics, Belgium) and used for IUI. The IUI was done as described by Vermeulen *et al*<sup>(11)</sup>. All the women instructed to be in supine position on the side of ovulating ovary for 30 minutes. Luteal support was started from the next day after insemination by using progesterone tablets (Duphaston® 10 mg; Solvay- Holland) twice daily for 2 weeks then a blood sample was obtained from the female to test for human chorionic gonadotropin ( $\beta$ -hCG). The pregnant women were followed for fetal gender determination from 16 week gestation on ward and the number of delivered babies was recorded thereafter.

### Statistical analysis

This was performed using SPSS (Statistical Package of Social Science; version 17.0 LED Technology, USA) and Microsoft Excel Work Sheet 2007). The results were expressed as

mean  $\pm$  standard error of the mean (SEM). Paired sample t-test and Chi-square were used to compare between the results depending on the nature of data. The differences between the values were considered statistically significant if the *P* was lower than 0.05<sup>(12)</sup>.

### Results

Table 1 showed different certain sperm function parameters before and after activation using albumin discontinuous density gradient of 7% and 17%. The mean of sperm concentration after *in vitro* activation was significantly (*P* < 0.05) decreased compared to before activation. There was a significant (*P* < 0.05) increase in active sperm motility grade A and grade B following *in vitro* activation compared to before activation. The percentage of sperm motility grade C and grade D was significantly (*P* < 0.05) decreased after activation than that of before activation. The percentage of morphologically normal sperm after activation ( $68.37 \pm 1.68$ ) was significantly (*P* < 0.05) higher than that of before activation ( $44.78 \pm 1.29$ ).

**Table 1. Sperm parameters of men semen whose spouses become pregnant after *in vitro* activation by discontinuous density gradient technique**

Parameters		<i>In vitro</i> activation by 7%-17% density gradient technique		T test value
		Before	After	
Sperm concentration ( $\times 10^6$ /ml)		$51.75 \pm 1.87$	$25.28 \pm 1.33$	10.37 *
Sperm motility (%)	Grade A	$10.15 \pm 1.26$	$57.70 \pm 2.05$	6.849 *
	Grade B	$31.36 \pm 1.44$	$32.58 \pm 1.18$	5.010 *
	Grade C	$29.44 \pm 0.72$	$6.71 \pm 0.68$	7.925 *
	Grade D	$29.05 \pm 1.55$	$3.01 \pm 0.52$	3.103 *
Morphologically normal sperm (%)		$44.78 \pm 1.29$	$68.37 \pm 1.68$	5.076 *
* ( <i>P</i> <0.05)				

Table 2 show the rate of pregnancy following IUI of 100 women involved in this study. There were twenty two out of one hundred women become pregnant (22%) and the seventy eight women did not get pregnant. The statistical analysis found a significant difference (*P* < 0.01) between them.

The total male babies was thirteen and the percentage of male sex selection was (76.64%), when one women pregnant with twins males babies, while only four female babies delivered which give 23.36% percentage (Table 3).

**Table 2. Pregnancy rate following IUI of 100 women**

Status	No.	%
Pregnant	22	22
Non- Pregnant	78	78
Total	100	100

Chi-square value = 9.644 \*\*

Sixteen pregnant women reach full term and delivered 17 babies representing 73.9%, while the remaining six sustained trimester abortion.

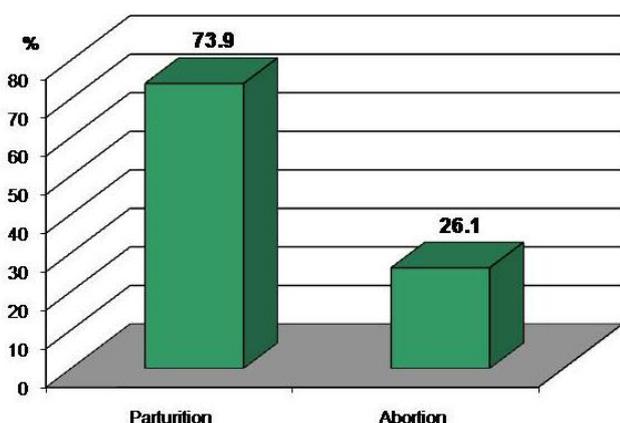
**Table 3. Distribution of Live birth according to gender**

Gender	No.	Percentage (%)
Male	13*	76.64
Female	4	23.36
Total	16	100

Chi-square value = 9.25 \*\*

\*One pregnant women delivered twins male, \*\* =  $P < 0.01$

There was a significant ( $P < 0.01$ ) higher percentage of term pregnancy compared to abortion out of the twenty two pregnant patients (Fig. 1).



**Fig. 1. Percentage distribution of pregnant status according to parturition and abortion**

### Discussion

The sperm preparation and activation *in vitro* by discontinuous density gradient technique using 7% and 17% albumin concentrations showed a significant improvement in sperm active motility grades A and B. This improvement in active sperm motility may be resulted from the effect

of the technique, which removed the seminal plasma, pus cells and agglutinated spermatozoa<sup>(13)</sup>. This (7% and 17%) albumin concentration for IUI was also used by Ericsson method for sex selection<sup>(14)</sup>.

In this work, there was a modification in Ericsson method by using discontinuous density gradient procedure. This technique is used in different fertility 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, immotile spermatozoa<sup>(15)</sup>. Therefore, the procedure of preparation by this technique improves both the sperm motility and percentage of morphologically normal sperms<sup>(16)</sup>. In addition to that, using human serum albumin for sperm preparation will enrich the medium with high amount of adenine monophosphate, which enhance sperm motility by fueling its energy and increase the number of highly active motile sperms<sup>(17)</sup>. Furthermore, albumin act as antioxidant in a way that it did not protect against DNA damage induced by NADPH, but is extremely effective at preventing DNA fragmentation arising from the suppression of glutathione peroxidase activity with mercapto-succinate<sup>(17)</sup>.

Out of the one hundred couples inseminated only twenty two got pregnant (22%), six ends with trimester abortion while the others got full term pregnancy. The pregnancy success rate following IUI was higher than that of IUI results in other studies<sup>(18-20)</sup> which range from 14%-21%.

The higher successful rate of pregnancy in this study could be attributed to the following facts: 1. the use of human serum albumin technique as centrifugation media, which was not used in those studies that may give lower pregnancy outcome<sup>(21)</sup>.

2. Preparation of the women for IUI in this study e.g. Ovulation induction and timing of insemination used) depend on criteria of previous study conducted in the Institute by Al-Dujaily and Abo-Regheef<sup>(22)</sup> which gave a high pregnancy rate too.

3. The average ages of women got IUI was 30 years old. It has been found that age of the patient is a significant influencing factor in the success of IUI<sup>(23)</sup>. In those women over the age of 40, the pregnancy rate following IUI is very low<sup>(24)</sup>.

**Gender selection outcome:** The pregnancy success rate was 22%; six cases ends with first trimester abortion, 75% of the delivered babies were male, while only 25% of the delivered babies were female babies. These results obtained match the results obtained by Dr. Ericsson method for sex selection<sup>(7)</sup>. The high incidence of male gender results can be attributed to the fact that discontinuous albumin density gradient result in clean fraction of concentrated sperms free from debris, round cells, immotile spermatozoa<sup>(25)</sup>. Moreover, Y chromosomes will be concentrated at the bottom layer of the centrifuge as the light Y chromosome can move rapidly down the different layers of albumin concentration in contrast to X chromosome which has higher molecular weight and hence cannot emerge easily from the high albumin concentration used in the discontinuous density gradient centrifugation. This gives high yield of male babies delivered to women inseminated using this method. However, the most important factor that may halt the increase in male sex selection in this study not more than 75% with percentage of 22% pregnancy rate is that all the couples are infertile which in turn interfere with the results of insemination by a sufficient number of male sperms<sup>(26)</sup>. As most of the semen samples were obtained from infertile men, this will increase the incidence of low number of male sperms before density gradient technique compared to fertile men semen.

On the other hand, out of the twenty two pregnant patients only two patients got twin pregnancy (9%) and one of them was aborted in the first trimester. The incidence of multiple pregnancies (twins) may result from fertilization of more than one oocyte due to ovulation induction programs. A major concern for couples undergoing any fertility treatment is the risk of

multiple pregnancies. For those couples undergoing ovulation induction with clomiphene alone, the risk of twins is about 11%<sup>(27)</sup>. For those couples treated with superovulation/IUI, the risk of twins is about 16%<sup>(28)</sup>.

The present study concluded that gender selection by Ericsson method is easy and effective procedure to enhance the desire of the infertile couples.

### Authors' contribution

The proposal design was suggested by Prof. Al-Dujaily. The performance of work and writing the manuscript were collectively done with Dr. Shighaf Al-Dahan.

### Conflict of interest

The authors declare no conflict of interest

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