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EFFECT OF OVULATION INDUCTION PROGRAMS ON THE SUCCESSFUL RATE OF GENDER SELECTION TECHNIQUE OF INFERTILE COUPLES.

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ABSTRACT

Back ground: There are many techniques used for sex selection. Ericsson method is the well- known to isolate Y sperm from X sperm. Modifications were done in many studies to increase the percentage of gender successful rate. However, in our knowledge there were cease studies correlated between gender selection and ovulation induction programs. **Objective:** To examine the effect of ovulation induction programs on gender selection following intra uterine insemination (IUI) for infertile couples. **Patients, Materials and Methods:** One hundred of infertile couples were included in the present study. They were randomly selected from the patients conducted the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. They were divided into four groups depending on the programs of ovulation induction Protocols were used. **Group one**, were orally administration Clomiphene Citrate (CC)only, **Group two**, were used Gonadotrophin injection. **Group three**, were used CC and Gonadotrophin. **Group four** (the control) were used free medicine and depending on their natural menstrual cycle .Then IUI was accomplished and pregnancy test was done after 14 days of insemination to detect the level of human chorionic gonadotropin. The gender of the fetus was detected by an ultrasound examination from sixteen weeks gestation on ward. **Results:** Total pregnancy rate following (IUI) for gender selection of infertile couples in all four groups were Twenty six out of one hundred Women who have become pregnant (26.0%). The distribution of Live birth babies according to gender using the four different programs of induction ovulation was 19 male babies with a percentage of male sex selection (82.6%) while only 4 female babies

were delivered with a percentage of (17.4%). **Conclusions:** The present study concluded that there was no effect of the programs of ovulation induction on the ratio of gender selection of infertile couple by IUI.

KEYWORDS: Ovulation induction, IUI, Gender selection.

INTRODUCTION

There were many histories of sex selection by different cultures world wild such as in Chinese, Egyptian and Greek cultures. The Chinese astrological birth chart, which is at a standstill used now days, is one of these.[1] However, since thirty five years ago, gender selection was accomplished in several ways either before implantation (sperm sorting) or after implantation of an embryo.[2] Assisted reproductive techniques (ART) such as intrauterine insemination (IUI) solved many male infertility factors which could only be solved by these techniques. Those spouses undergo IUI with ovulation induction medications generally have a 25-30% chance of becoming pregnant in any given cycle. Therefore most couples will be pregnant, but some couples will require more than one cycle.[3,4]

Gender selection technique was patented in the 1970s by Ronald Ericsson. This technique is based on the idea that Y-bearing (boy-producing) sperm are lighter and swim faster than the X-bearing (female-producing) sperm. The Ericsson Method sets out to divide the heavier, slower sperm-producing a girl from the lighter, faster-swimming sperm-producing a boy.[5,6,7] Many studies were developed this procedure in the sperm manipulation *in vitro* and increase the percentage of gender selection such as the studies of Al-Dujaily and Al-Dahan[8] and Al-Dujaily and Al-Jubori.[9] However the effect of using the programs of ovulation induction on gender selection following ART not studied yet. Therefore the objective of this study is to examine the effect of ovulation induction programs on gender selection following IUI for infertile couples.

PATIENTS, MATERIALS AND METHODS

This study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, since September 2015 till May 2016. One hundred infertile couples were included in the current work. Couples were carefully selected through detailed history (*in vitro* fertilization indicated cases were exclude) viewing all previous investigations .Men with normozoospermia and mild male factors infertility were included (those with mild oligozoospermia, mild asthenozoospermia, and those with

leukocytospermia). The excluded couples were sever male factor infertility and women polycystic ovary ,endometriosis, tubal occlusion and others.

Ovulation induction

Different induction protocols used for different female patients.

1-Colmid 50 mg tablet (Clomiphene citrate 50 mg, Aventis, France from(50-150) mg/day for 5 days starting from day 2 of the menstrual cycle.[8]

2-Gonadotrophic were used alone. Such as Follitropin alfa (r-hFSH ,75 IU of Gonal-F) or Puregon Inj. 50mg that give at day 3 , 5 and 7 depending on the woman ovaries response.[8,9]

3-Gonadotrophins and Clomid: The women of this group have 2-3 repeated doses of 75 IU/day of injectable gonadotropins were added to Clomiphene citrate tab.[9]

4-Natural cycle group: The another women group was considered control group and no medicine used but depends on natural cycle included in this study. (Ovitrelle 250mg, Merck Serono S.P.A. Italy) given to trigger ovulation, Insemination done usually 34-36 hours after hCG injection.[10,11]

2. *In vitro activation* technique for gender selection

- Male examination and Investigation

The husbands who included in this study were examined by a consultant urologist in the Institute. Semen sample was obtained via masturbation after an abstinence period of 3-5 days[12], collected directly into a clean, dry and sterile disposable wide mouth container in a special allocated room for this purpose in the Institute. The sample was transported to the semen examination laboratory immediately and allowed to liquefy in an incubator at 37ºC. After complete liquefaction, the semen was analyzed by a macroscopic and microscopic examination using the standardization of WHO (1999).[13]

***a- In vitro* sperm activation by swim up (Simple layer).**

One ml of warmed Hams-F12 medium was added gently and carefully to overlay liquefied semen in a sterile test tube then left for 10-30 minutes in CO_2 incubator at 37ºC.

b- Discontinuous density gradient centrifugation

Discontinuous density gradient centrifugation technique was prepared using percoll concentration of (40% - 80%). One ml from the superficial part of the Hams-f12 media of the prepared test tube was aspirated and added to overlay another prepared warmed 80% and 40 % of the percoll solution respectively in a sterile grade 15ml conical centrifuge tube

then we put this prepared tub in the centrifuge at 4000 for 20mins as described by Al-Dujaily and Al-Jubori.[9]

C-Preparation of Albumin

Albumin 17% and 7% were prepared by using human albumin 20% (Biotast, Pharma, GmbH, Germany was prepared as described by Al-Dujaily and Al-Dahan.[8]

2. Protocols of semen preparation using Ericsson method.

D- Intra- uterine insemination protocol

The female partner was prepared for the insemination process, by asking her to empty her bladder and to lay down in a lithotomy position on the couch. A warmed and lubricated Cusco speculum was introduced gently and slightly down word to expose the cervix which was washed with saline or few drops of Hams-F12 media. The cervical mucus was removed by a small piece of cotton and the cervix was prepared by using aseptic techniques. The loaded IUI catheter passed through both the external and internal Os using a marker guide line for the correct placement. The sperm preparation (0.3-0.5ml sperm suspension) was gently inserted into the uterine cavity. The speculum gently removed and the patient was instructed to spin on the side of ovulating ovary for 30 minutes.[9]

E-The follow up of the female partner after insemination.

1. Luteal phase support

Luteal phase supplementation started from the next day after insemination using a 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 a week or vaginal route (Crinone 8%) daily for 2 weeks.[14]

2.Follow up for pregnancy and pregnant female

A blood sample was obtained from the female after 2 weeks from the insemination to test for beta chain of human chorionic gonadotropin (B- HCG) level. If the result of the test was positive, the patient was undergoing a follow up and careful antenatal care by an obstetrician with regular visits.

An ultrasonography was done to confirm viability of intra uterine pregnancy, fetal growth and development, and at 16 weeks of gestation an ultrasound was confirmed for the fetal sex

determination[14], while women with a negative pregnancy test, a new cycle of intra uterine insemination was done.

Statistical analysis.

A statistical analysis was performed using SPSS (statistical package of social science, version 21.0 LED technologies, USA) and Microsoft Excel work 2010 for figures. The results were expressed as mean + standard error (SE). Students t-test was applied to differentiate between two groups .Analysis of variance (ANOVA) was used for more than two variables .When p value reached the significant level, the least significant test (LSD) was performed. Chi square was done for the non -parametric values. The differences between the values were considered statistically significant if the P value was lower than 0.05($P<0.05$).[15]

RESULTS

1: Programs of ovulation induction of the present study.

The study involved four ovulation induction programs namely; by using CC, the number of couples was 25, the second program was by injection of Gonadotropins, the number of couples was 28 and the third program was by mixing both CC and Gonadotropins, the number of patients was 35 and the last program was natural cycle, the number of couples was 12 as shown in table (1).

Table (1): Programs of ovulation induction.

Woman's groups	Program of ovulation induction	Number =100	Percentage %
1	Clomiphene citrate.(CC.)	25	25.0
2	Gonadotropins.(Gonad.)	28	28.0
3	CC./Gonad.	35	35.0
4	Natural cycle	12	12.0

2-Ultra sound finding of follicular size and endometrial thickens prior to Human chorionic gonadotropin injection of infertile couples involved in gender selection study.

Table(2) shown, the follicle size and endometrial thickness in women ,whose husband semen activated *in vitro* by using density gradient technique and albumin technique for gender selection. The mean of follicular size in the four groups involved in gender selection techniques was revealed no significant ($P>0.05$) different between them. There was no significant ($P>0.05$) differences in the mean of endometrium thickness between the four groups of ovulation induction programs in women underwent gender selection (CC= 8.15 ± 0.324, G=8.82 ± 0.1 CC and G 8.7 ± 0.116, natural cycle= 8.5 ± 0.177, respectively).

Table (2): Ultra sound finding of follicular size and endometrial thickness prior to Human chorionic gonadotropin injection of infertile couples involved in gender selection study*.

Parameters Ultrasound	CC	Gonadotropins	CC/Gonad	Natural cycle
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Follicular size	20.9±0.327	19.9±0.497	20.7±0.277	20.9±0.353
EM	8.15±0.324	8.82±0.188	8.7±0.116	8.5±0.177

P>0.05

3-Total pregnancy rate following IUI for gender selection of infertile couples in all four groups.

The total pregnancy rate in the present study for gender selection was 26 spouses out of 100 (26.0%).The number of women who completed the pregnancy were 23 (88.5%) and only 3 aborted(11.5%).The study recorded 19males (82.6%) and 4 females(17.4) as shown in table (3).

Table (3): Total pregnancy rate following IUI for gender selection of infertile couples in all four groups.

No . of patient	No. pregnant patient		No. non pregnant	
100	26	26.0%	74	74%
No. complete pregnancy		No. abortion		
23	88.5%	3	11.5%	
Male		Female		
19	82.6%	4	17.4%	

4: Pregnancy rate following intra –uterine insemination for gender selection of infertile couples using four groups of induction ovulation programs.

The pregnancy rate(PR) following IUI for gender selection of infertile couples using Clomophin Citreat (groupI) was 6 out of 25 (24%) patients while the number of non pregnant was 19 (76.%0) . Wherease the PR in group II of patients using gonadotrophin under went IUI for gender selection was 7 (25.0)out of 28(28%). While 21(21.0)were not pregnant. The PR in groupIII were using CCand gonadotrohpin was 10(26.3) out of 35 (35 %0) couple with 25 (73.3%). The pregnancy rate of the fourth group of anatural cy was3(25%)out of 12 and the non pregnant were 9 (75 %). There was a significant (P<0.01) improvement in PR of group III that used CC and gonadotropine compared to the other three groups as shown in table (4).

Table (4): Pregnancy rate following intra –uterine insemination for gender selection of infertile couples using four groups of ovulation induction.

Programs of ovulation induction		*No.*	*Percentage %*
Group 1(clomiphene-citrate)	Pregnant	6	24.0
	Non pregnant	19	76.0
Group 2(Ganado trephine)	Pregnant	7	25.0
	Non pregnant	21	75.0
Group 3 CC/gonad.	Pregnant	10	26.3*
	Non pregnant	25	73.7
Group 4/Natural cycle	Pregnant	3	25.0
	Non pregnant	9	75.0

***P<0.01 High significant compared to other ovulation induction programs.**

5: Illustration the successful gender selection following complete pregnancy.

In the group I the number of women who completed the pregnancy was 5(83.3%) out of 25with 4(80%) males and 1(20%) females. The percentage of successful gender selection was (80%)**.** In group II, the no. of women who completed the pregnancy was 6(85.5) out of 28 with 5(83.5) Male and 1 (16.7%) Female. The percentage of successful gender selection was (83.3). In group III, the no. of women who complete pregnancy was 9 out of 35 with 7(77.8) Male and 2(22.2) Female. The percentage of successful gender selection was (77.8 %).In group four the number of women who couplet the pregnancy was 3(100%) out of 12 all of them were male (100%) as shown in table (5).

Table (5): Illustration the successful live birth and the gender following IUI program of infertile couples.

Groups	*Pregnancies*		*No and%*	*Gender no.%*	
	Total	*Aborted no.*	*Complete*	*Male*	*Female*
Group I (CC)	6/25	1	5 (83.3%)	4(80%)	1(20%)
Group II (Gonad)	7/28	1	6(85.7%)	5(83.3)	1(16.7%)
Group III (CC+Gonad)	10/35	1	9(90%)	7(77.8%)	2(22.2%)
Group IV (natural)	3/12	0	3(100%)	3(100%)	0(0.0%)

DISCUSSION

Comparison between the four different groups of ovulation induction programs with the pregnancy rate

The results of the four programs of induction ovulation for IUI of gender selection of infertile couples which have been used in this study found that the mixing of CC with Gonadotrophin in group three .The highest ratio of pregnancy was in program of third group which was 10 women get pregnancy out of 35 women with percentage of (26.3 %). Consequently, the results of pregnancy by IUI were significantly increased in the third group who they were treated by mixing two types of ovulation induction drugs (CC + Gonadotrophins) as the percentage of pregnancy was (26.3%) compared to other three groups of the induction ovulation programs. This observation can be explained by strong positive effect of the above two drugs on the endometrial thickness and the follicular size that reached as indicators of good oocyte maturity when used together comparing of each drug used alone leading to normal implantation and each of these medications have its properties in ovulation induction result .It has been postulated that CC was a selective estrogen receptor modulator (SERM) of the triphenlethylene group that has become the most widely prescribed drug of ovulation induction to reverse an ovulation or oligo ovulation.[16] Clomiphene is useful in those who are infertile due to an ovulation or oligo ovulation.[17] Thus , this mean that it increase the number of follicles that entered the stage of recruitment and selection with increasing the follicular size and endometrial thickness .The current study could be attributed to the fact that all women were under good ovulation induction medications and subsequently good mature follicles giving effective endometrial thickness leading to a successful pregnancy rate.[18]

- Pregnancy following IUI

The data of this study recorded a high pregnancy rate following *in vitro* activation using ovulation induction program of CC and Gonadotropin which is (26.3%) compared to other programs (program 1= 24.0%, program 2= 25.0%and the natural cycle 25.0%). The high percentage of pregnancy was similar to a previous study done by Al-Dujaily and Abo-Rhgeef[19], when they recorded a pregnancy rated 37% and increases the successful fertilization, embryonic development, and implantation and pregnancy rate. The positive correlation between the high percentage of MNS and fertilization rate, embryonic development and pregnancy rate by other studies[20,21] have been emphasized. It seems that the selection of couples with minor male infertility causes (mild asthenoteratozoospermia) may influence the positive results of assisted reproduction programs.[22,23] Consequently, optimum male and female fertility potential criteria will lead to a high and successful pregnancy rate.[19]

- Gender selection outcome illustration the successful gender selection following complete pregnancy

This high percentage in male selection is attributed to the mixing of different techniques for sperm preparation.[24] All used in gender selection not to the programs of induction ovulation.This result matched the results obtained by Dr. Ericsson, method for sex selection[7], Al-Dujaily and Al-Dahan, result[8] and AL-Dujaily and AL-Jubori.[9] The high incidence of male gender results can be attributed to the fact that the simple layer, discontinuous density gradient with albumin techniques result in a clean fraction of concentrated sperms free from debris, round cells, immotile spermatozoa. Moreover, Y chromosomes will be aggregated as a pellet at the bottom layer of the test tube as the light Y sperm can move faster through the layers of albumin concentration in contrast to X sperm which has a higher molecular weight and hence cannot emerge easily from the high albumin concentration used following discontinuous density gradient centrifugation.[23] This gives high yield of male babies delivered to women inseminated using this method.

The present work was noticed that there was no significant relation between the type of ovulation induction programs and the percentage of pregnancy with male so the medications of ovulation induction not affect the gender male selection. But these drugs effect the percentage of pregnancy rate following intra –uterine insemination.

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