Correlation between in vitro sperm preparation techniques, endometrial thickness, hormonal profile and successful pregnancy rate following IUI: retrospective and prospective study

Saad S. Al-Dujaily and May Abdul-Kareem Aburgheef
Clinical Reproductive Physiology, High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad-Iraq Corresponding Author. e-mail:aldujaily8@yahoo.com

Abstract:

Background:
The intra uterine insemination (IUI) still an easy, simple and effective method to overcome the infertility that resulted from different causes. Although the equipments and methods of assisted reproductive technologies (ART) were highly developed, the percentage of successful IUI live-birth not exceed 20- 30%.

Objective:
The present study was aimed to elucidated the cut off values of *in vitro* sperm preparation technique, follicular number and size, endometrial thickness and hormonal profile that lead to successful pregnancy rate (PR)following IUI.

Materials and Methods:
In retrospective study, one hundred cases of infertile couples were in who were became pregnant following IUI at the Institute of Infertility Diagnosis and Assisted Reproductive Technologies, through the period from January 2007 to January 2010. Depending on the results of retrospective study, IUI was achieved for 100 infertile patients between August 2010and June 2011. The mean of significant prognostic variables were measured in both studies namely: age, type of infertility, type of sperm activation techniques, the protocol of ovulation induction medicine, the mean of hormonal status of Luteinizing Hormone (LH), Estradiol(E_2), and number with diameter of follicles, endometrial thickness a cycle day before hCG injection

Results:
The protocol of ovulation induction in IUI cycles by using clomiphene citrate(CC) and recombinant gonadotropin (rFSH) was significantly($p<0.05$) improve the IUI results compared to CC alone or CC with Pergonal®. The mean values obtained by ultrasonography namely; two follicles, and dominant follicle of 19.5mm in size with endometrium thickness of >9.5mm were gave significantly successful IUI outcome compared to other values. In prospective study similar results were obtained ,therefore the overall pregnancy rate was 37% per cycle.

Conclusion:
The calculated data of successful IUI outcome from retrospective study gave a best pregnancy rate in prospective study which was 37%.Consequently, these results can be considered as guideline for physician who interest in assisted reproduction to have a success in IUI.

Key words: IUI, *in vitro* activation, endometrium thickness, pregnancy rate

Introduction:

Infertility is defined as a year of unprotected intercourse in the fertile phase of the menstrual cycles in women under 35 years, or after 6 months in women 35 years or older without achieving pregnancy[1].The origin of infertility is similarly due to male and female factors resulted from multiple causes. Female factors account for 38% of infertility. Male factors account for 20% of infertility. Male and female factors combined cause 27% of fertility. The etiology is unknown in 11%, and other causes are identified in 4%[2,3]

Accuracy of diagnosis is the most important factor to solve the infertility problem[4]. Then the treatment can be determined by medicine and/or assisted reproductive technologies[5]

Intrauterine insemination is a method of ART recognized to be effective and inexpensive and can be offered by both secondary and tertiary fertility centers and to entail relatively few restrictions. It is not as invasive as IVF and allows fertilization to occur within the fallopian tubes and therefore is generally acceptable to most religious groups[6].

To improve the results of IUI, the selection of couples and determination of criteria before starting the IUI program until the detection of pregnancy must be noticed. Therefore, the present study is designed to find out the cut off values that led to pregnancy following IUI. Then these values will be applied in prospective study to found out the PR following IUI. Thus,this work will include retrospective and prospective studies to 1-Examine the effect of *in vitro* sperm preparation techniques on IUI outcome.2-Elucidate the correlation between endometrial thickness and hormonal profile before hCG injection and their correlation with PR following IUI.

Materials and Methods

This study was conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University – Baghdad through a period from July 2010 to April 2011. The proposal research is designed to evaluate two categories .

1-Retrospective study:

The study calculate one hundred cases of infertile couples who were became pregnant following IUI in the Institute ,through the period between January 2007 and January 2010. The calculated data will focus and concern on the circumstances namely; techniques of *in vitro* sperm activation (simple layer -centrifugation), hormonal profile, endometrial thickness(ET), size and number of dominant follicle(s) all in which gave best results leading to pregnancy

2-Prospective study:

The prospective experimental study included 100 infertile patients who are attending Consultant Clinic at the Institute between Augusts 2010 till June 2011.Depending on the retrospective results, IUI was done by using the criteria of successful sperm preparation technique. Optimum measurement of follicular size, number and ET by using the ultrasonography. The level of reproductive hormones (LH,FSH and Estradiol) was determined in cycle day 2-3 of menstruation and before the injection of hCG.

The IUI was done when the values of ET, Hormonal assay and follicular size before hCG are similar to the calculated data in the retrospective study to found out the PR.

Couples who were not fit for IUI were excluded from this study (such as severe oligozoospermia in men and tubal agenesis in women)

2. Initial investigation

All couples were asked to complete a self-assessment form attending their first visit. The investigation was performed to assess the normal menstruation and: hormonal assay (7). In retrospective and prospective study, hormonal tests were performed at day two of cycle these included FSH, LH, estradiol (E2).For prospective study levels serum LH and E2 were measured at cycle days 9- 11 to predict ovulation

-Ultrasonography Monitoring: In this study, it had been routinely performed a vaginal scan in the early follicular phase (Cycle day6) to excluded PCOs, previous Cyst and antral follicle. At Day 9 (Cycle day6) follow up follicle(s)diameter, and response to treatment. At cycle day12, dominant follicle size with number and endometrial thickness were assessed(9).

3. Ovulation Induction

In prospective study, the protocol used for ovulation induction was clomiphene citrate (Clomid® tablets, 50 mg, Aventis, France) two times daily for 5 days from day3 of menstrual cycle to day7(10). Also Gonal-F® from day 3 of menstrual cycle was injected and the dose was adapted depending on the ovarian response to the treatment, till maturity of follicle,(11).

All the males included in the study were examined by male infertility consultant in the Institute and standard semen analysis was performed according to the WHO guidelines(12).

4. *In vitro* activation technique:

Simple layer(Migration -sedimentation) procedure was performed for the semen samples that involved in the prospective study as described by Al-Dujaily(13).

5-Intra-uterine insemination technique

Intra-uterine insemination was performed when two ovarian follicles reach a measurement of (19.5mm) and endometrial thickness(9.5mm) were reported by ultrasonography with serum E2 level (750pg/ml) and following 36 -40 hours of human chorionic gonadotrophin (hCG, Ovitrelle

® 250 mg =6500IU) injection. About 0.5 -0.7ml of activated sperms were loaded in a syringe and then attached with IUI catheter.Insemination was performed according to Elnashar,(10). The diagnosis of pregnancy was done either by biochemical analysis (the detection of hCG in the blood after 2weeks of insemination) or by ultrasound examination,(14,15) .

6- Statistical analysis:

Statistical analysis was performed using SPSS (Statistical Package for social Science; Version (16.0) and Microsoft Office Excel 2007. Numeric data were expressed as mean ± SE. Nominal data were expressed as frequency and percent. Numeric data were analyzed using student t-test. Nominal data were analyzed using Chi-square test,(16) .

Results

1. Characteristics of the retrospective study

1.1. Assessment of *in vitro* sperm activation techniques used in the retrospective study

The number and percentage of two *in vitro* sperm activation techniques namely; The simple layer and the centrifugation techniques) that were used in IUI and resulted pregnancy in retrospective study:1-simple layer percentage (52%) 2-centrifugation (11) percentage (11%)3-by use of both methods (37)with percentage (37%).

1.2.Hormonal status of women included in the retrospective pregnant study (cycle day2)

Table (1) shows hormonal status of pregnant women involved in retrospective study.The mean of the FSH level was (4.1 +0.349) mIU/ ml with a range between 0.90 to 20.00 mIU/ml. The mean of LH level was (11.6+ 0.342)mIU/ml (ranged between 1.00 to 22.2 mIU/ml).The mean of serum estradiol level was (136.7 +2.895) pg/ml (ranged between 40 to 281.00 pg/ml).

1.3. Characteristics of the retrospective study of pregnant women, the day before hCG injection.

The endometrial thickness in the pregnant females ranges from (6-12.60) mm with a mean thickness of (9.46 +0.373) mm. The mean size of Graffian (dominant) follicle was (19.5+ 0.998)mm (ranged between 17.00 to 23.00 mm). The number of Graffian follicles of successful pregnant women was between 1-3 follicles with a mean of (2+0.062) as shown in table(2).

1.4. Ovulation induction program in the retrospective pregnant study

The ovulation induction program used by the pregnant female included in the retrospective study: 1- natural cycle (without induction) was (18) with a percent (18.%), 2-Ovulation induction by Clomid® only was (25) with a percent (25.0%) 3-Ovulation induction with Clomid® and Gonal .F® was(47) with percent(47.0%), 4-Clomid®and pergonal® was(10) with percent (10.0%).

2.Characteristics of the prospective study

2.1. *In vitro* sperm activation using simple layer technique

Table (3)showed the effect of the simple layer) migration-sedimentation technique) on human sperm parameters. for men where their spouses having the previous parameter (retrospectively). The sperm concentration was significantly (P<0.05) lower (30± 1.41) than that of before activation (55 ±0.43).The progressive motility grade A was highly significant(P<0.01)increased (45± 1.73) after *in vitro* activation compared to before activation(19.4±0.04).Progressive motility grade B (40 ±1.51) were significantly (P<0.05) increased after *in vitro* activation compared to before activation(31.01± 0.48). A high significant (P<0.01) improvement was recorded in the percentage of morphologically normal sperm following *in vitro* activation (70± 2.4) compared to that before activation(40.7± 0.4) .A significant(P<0.05) decrease in the number of round cells was recorded after activation compared to before activation (Table -3).

2.2. Levels of serum LH and Estradiol Hormones in cycle day before the hCG injection in the prospective study.

In table(4) there was a significant (P<0.05) differences in the level of LH (15.63 ± 3.63 mIU/ml) in cycle day before the injection of hCG in women were got successful pregnancy (N=37) compared to the level of LH (11.62± 1.291 mIU/ml) in the non-pregnant women group (N = 63).

Level of estradiol hormone (pg/ml) revealed a significant (p<0.05) increase before the injection of hCG in the pregnant women group (787.74 ±17.323) compared to the non-pregnant women group (549.12 +27.409).

2.3. Percentage of pregnancy in IUI in the prospective study

Intra-uterine insemination was done for 100 infertile women who have the cut off values similar to retrospective study successful results. The mean of endometrial thickness was (9.5mm), the size of dominant follicle was (19.5mm) and the number of follicles was (2),using CC and G.F only as an ovulation induction protocol. Semen samples of their spouses was prepared only by simple layer) migration -sedimentation) activation technique. The result of pregnancy rate was 37% after IUI as shown in figure 1.

Table- 1: Hormonal status of pregnant women included in retrospective study(cycle day 2)

Hormones	Minimum	Maximum	Mean	SD	SE
FSH	0.90	20.00	4.1	3.19	0.349
LH	1.00	22.20	11.6	4.60	0.342
Oestradiol	40.00	28k1.00	136.7	24.71	2.895

No. women=100

Table 2: Characteristics of endometrial thickness, size of dominant follicle, and number of dominant follicle in the retrospective pregnant study (n =100),the day before hCG injection

Characteristic	Minimum	Maximum	Mean	SD	SE
Endometrial thickness	6.00	12.60	9.5	1.05	0.373
Size of dominant follicle	17.00	23.00	19.5	1.25	0.998
Number of follicles	1	3	2	0.63	0.062

Table 3- :*In vitro* activation by using simple layer technique

In vitro activation		Sperm concentration (Million/Ml)	Sperm motility				Morphologically normal sperm (%)	Round cells (cell/HPF)
			A	B	C	D		
Before Activation	Mean	55.9	19.4	31.01	12.5	29	40.7	17.6
	SE	0.43	0.04	0.48	0.23	0.25	0.4	0.31
After Activation	Mean	30.2*	45**	40*	10	5**	70**	0.00
	SE	1.41	1.73	1.51	0.617	0.514	2.43	0.00

*P<0.05 Significant
**P<0.01 High significant
Student>s t-Test

Table 4: Comparison levels of LH and Estradiol hormones between pregnant and non pregnant women in the day before hCG injection in prospective study

Characteristics	Group 1 Successful pregnancy N = 37	Group 2 Failure of pregnancy N = 63	P	Significance
LH (prospective)	15.63+3.63	11.62+1.291	0.005	Significant*
E2 (prospective)	787.74+17.323	549.12+27.409	0.005	Significant

No women=100
Student>s t-Test

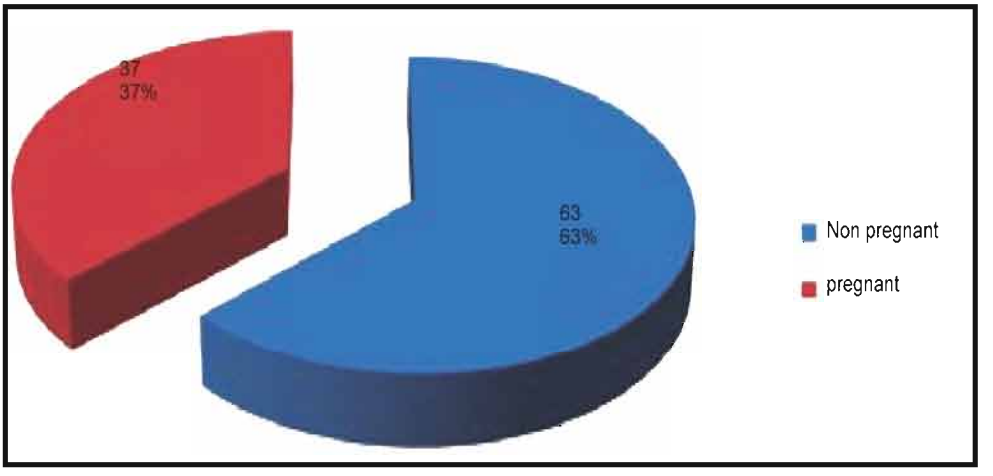

Figure - 1: pregnancy rate by Intra Uterine Insemination in prospective study

Discussion

The mean age of the whole partners shared in this study was (30.20± 4.60 years). The majority of pregnancies happened among the women their age <35 years old and in the first treatment cycle. The success of IUI also declined with increase of patient age, which is in congruity with other reports,[17]. The age-related decline in female fertility is probably due to a combination of reduction in oocyte number and quality[17] ,diminished ovarian reserve, decline in granulosa cells function, reduced endometrial receptivity,[18]

In retrospective study and then in prospective study, simple layer technique was used as the technique for *in vitro* sperm activation in which the PR was reached 37% after IUI. This procedure still remains the standard technique for patients with normozoospermia and female infertility. The technique distinguished by a very high percentage (>90%) of motile sperm, preferred enrichment of morphologically normal spermatozoa as well as the absence of other cells and debris[19].

Furthermore, The data revealed a highly significant (P<0.01) increased in the percentage of the progressive motility grade A after *in vitro* activation compared to before activation.Progressive motility grade B was significantly (P<0.05) increased after *in vitro* activation compared to before activation, this result is similar to the results of other studies,[20] It has been mention that the increase in the PR from 17.1%to 30.4% if the active sperm motility grade A exceeded 20%[20].

Measurements of FSH and E2 of women in menstrual cycle, at day 2 and day 3 combined with maternal age, are useful for predictors of pregnancy outcome. These measurements are standard practice for predicting oocyte quality and the likelihood of conception in assisted reproductive technologies (ART),[21].

The result of the present work indicated a significant increase in the level of estradiol hormone in cycle day before hCG hormone injection in pregnant women) compared to non-pregnant women. Although both groups had the same ultrasound characters (2dominant follicles measured 19.5mm), follicles are believed to contain a mature oocyte when they are 20 -24 mm and follicle size increases by 2-4 mm after the start of the LH surge, this means that hCG should be given when the largest follicle is 19.5-20 mm,[21],however, some of mature follicles in non-pregnant women didn't contain oocytes and the E_2 level was decrease [22].

The results of this prospective study identified that hCG must be administered when the serum estradiol level reaches 300- 420 pg/ml per follicle >19.5 mm in diameter, 700 -800 pg/ml per two mature follicles on the day before hCG administration. the pregnancy rate per cycle varied according to the E_2 concentration [23].It has been noticed that PR was 14.2% per cycle when E2 level was <500 pg/mL on the day of hCG administration[24], 16.2% per cycle when the E_2 was between 500 and 1000 pg/rnl, and lastly, 22.1% for over 1500 pg/rnl,[25].

It has been recorded that ultrasound should be used for timing of hCG injection to avoid complications, relies on most for the decision to increase, decrease, or` stop the gonadotropin.[26].

The present retrospective study identified that the mean of endometrial thickness was (9.5) in the day before hCG injection. There was a positive linear correlation between endometrial thickness and pregnancy rate[27, 28]. The study stated that thickness can be utilized as an indirect indicator for endometrial receptivity Ultrasonographic examination was routinely performed in ART treatments because of its accurate evaluation and being noninvasive detection. Indeed, both endometrial thickness and endometrial pattern have been regarded as prognostic parameters for successful pregnancy . It has been found that injection of hCG induced disappearance of the triple line pattern, probably representing normal physiological transformation from the proliferative to the secretory phase[29].

The current study revealed a significant relationship between the ovulation induction protocol and pregnancy rate. t has been reported that there was a significant improvement in PR(26.21%) by using CC+ rFSH protocol compared to (15%) PR following hMG protocol,[30].

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