



How Frequent Multi Follicular Response in Intra Uterine Insemination Cycle Leads to Multiple Pregnancy in Prolong Primary Subfertile Patient?

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

Received: 20/10/2023

Accepted: 27/12/2023

Published: 02/01/2024

ABSTRACT

Previously published studies on multi follicular studies are not consistent. Intra uterine insemination (IUI) is a good treatment option for limited resource countries because of its cost effectiveness and easy accessibility in remote areas. We aimed to identify pregnancy determining factors of IUI following controlled ovarian stimulation among educated sub fertile women in Bangladesh. A cross sectional retrospective study was conducted at Fertility Center of Evercare Hospital Dhaka, Bangladesh from January 2016 to December 2018 where 518 IUI cycles performed after taking written consent from participants. A total of 426 couples medical records were analyzed on the basis of inclusion and exclusion criteria. ovarian stimulating agent like clomiphene citrate tablet and human menopausal gonadotropin (HMG) subcutaneous injection alone or combined has been given to every women under this study. While at least one follicle diameter reached 18 mm then intramuscular Human Chorionic Gonadotropin (HCG) was given and IUI procedure was performed after 36 hours later. While analyzing the data a higher mean \pm SD was observed in pregnant groups than non-pregnant one regarding women's age, BMI, their husband's initial total motile sperm,

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inseminated harvested sperm and endometrial thickness though duration of married life was more in non-pregnant but these were not statistically significant. But the number of mature follicle was significantly higher in pregnant woman ($P < 0.001$). More than three follicle yield highest pregnancy than single or double follicles. Multi follicles showed a gradual decrease with age. A higher IUI was observed in woman with normal ovarian reserve. We propose that, IUI could be an effective therapeutic procedures for women with primary subfertility and could aid as an effective assisted reproductive technology in medical science.

Keywords: Intra uterine Insemination; primary subfertility; multiple pregnancy.

1. INTRODUCTION

As a cost effective therapeutic procedure in fertility management, intra uterine insemination (IUI) is used where the fertilization process of an ovum facilitated simply by placing a good number of purely motile sperm into the uterine cavity [1]. It can be performed in the natural cycle, that means IUI procedure is performed on the single mature follicle that release a mature oocyte after ovulation although success rate of the natural cycle is pretty low as 8-10%. Another methods called controlled ovarian hyperstimulation (COH)-IUI where ovulation inducing drugs are used to produce high number of mature follicle that gives higher opportunity of fertilization for inseminated sperm and pregnancy rate is much higher than natural cycle IUI. Multiple variables like ovulation, patency of fallopian tube, uterine malformation, age, BMI and husband sperm count are determinates of IUI with a wide range of 8-22%. However, irrespective of age and indications of IUI, overall pregnancy rate in COH induced multiple mature follicle increased to 6- 8% when compare to single mature follicle response [2]. Though the rationale COH-IUI are established by proven success from many researches, the researchers still could not established the optimal mature follicle number for a single live birth. Nevertheless, multiple mature follicle increased the risk of multiple pregnancy, maternal and fetal morbidity including higher abortion rate, reduced live birth rate, pre term delivery and low birth weight [3,4].

Though there is no general agreement regarding optimal follicle number for a successful IUI, some studies recommend to cancel IUI program for more than three mature follicle. Surprisingly, some investigators reported younger women (<32 years) and higher estradiol level on the day of HCG are the indicators of multiple pregnancy instead of follicle numbers. Two earlier studies conducted by researchers also determined the effect of follicle number in both live birth rate and pregnancy rate and the relationship between

outcome of COH-IUI and number of mature follicles [5,6].

But, previously published studies on multi follicular studies are not consistent. IUI is a good treatment option for limited resource countries because of its cost effectiveness and easy accessibility in remote areas. Effectiveness of COH induced high order follicle number with IUI has been reported by many investigator. The present study aimed to identify pregnancy determining factors of intrauterine insemination following controlled ovarian stimulation among educated sub fertile women in Bangladesh.

2. MATERIALS AND METHODS

2.1 Study Design

This is a retrospective research was performed at Fertility Center of Evercare Hospital Dhaka, Bangladesh from January 2016 to December 2018 of 518 IUI cycles. A total of 426 couples medical record were analyzed where the women had received IUI treatment by using their husband's sperm. A written informed consent was obtained from all the participants. All the women performed at least one year regular unprotected sexual intercourse with their husband before proceeding to the fertility treatment. Primary diagnostic workup was obtained before controlled ovarian stimulation (COS) that included relevant medical history, physical examination, trans-vaginal ultrasound, hormonal level, hysterosalpingogram (HSG) or laparoscopy, basic semen analysis for husband for every infertile couple to establish primary cause of infertility. The hormonal profile such as serum follicle stimulating hormone (FSH), serum luteinizing hormone (LH), serum estradiol, serum anti-müllerian hormone, serum prolactin, serum thyroid stimulating hormone (TSH) and were analyzed on the third day of menstrual cycle. Serum progesterone level was evaluated on 21th3

day of menstrual cycle and trans-vaginal ultrasound (TVS) was performed on the 12th day of menstrual cycle to diagnosed ovulation status. Male factor fertility capacity was evaluated by basic semen analysis report as per guideline of the WHO semen analysis manual [7]. The following inclusion and exclusion criteria were used:

Inclusion Criteria: Women between 20 and 44 years with at least one mature ovarian follicle, optimal endometrium thickness (more than 7 mm) on the day of Human chorionic gonadotropin (HCG) trigger, on the day of trigger with patency of one fallopian tube and husbands with normal sperm count according to the WHO semen analysis guideline [7].

Exclusion Criteria: (a) Woman with bilateral fallopian tube block. (b) Presence of any uterine pathology that affect implantation (polyp, fibroid, adenomyosis). (c) suboptimal endometrium (less than 7 mm) and. (d) husband and frozen sperm insemination.

All the women had taken ovulation inducing agent for follicular development: clomiphene citrate (CC) alone or human menopausal gonadotropin (HMG) alone, or HMG combined with CC. Choice of drug was based on each patients characteristics that included previous drug response, women's age, duration of infertility and hormonal profile. Clomiphene citrate was taken orally from the Day 2 to day 6 of menstruation and follicular development status was checked by TVS on the 12th day of menstruation. HMG was administered subcutaneously from day 2 to day 7 of menstruation and TVS for follicular monitoring done the 8th day of menstruation. For combined oral and injectable drugs, clomiphene citrate was taken from day 3 to day 7 of menstrual cycle and HMG injection administered subcutaneously from day 5 to day 9 of menstrual cycle, and folliculometry performed on the 10th day of menstrual cycle. The dosage of drug adjustment was made based on findings of the Serial trans-vaginal folliculometry. When at least one follicular diameter reached at 18 mm then Human Chorionic Gonadotropin injection was given through intramuscular (I/M) route. And also measured endometrial thickness during serial folliculometry. Intrauterine insemination procedure was performed after 36 hours of the Human Chorionic Gonadotropin injection.

Sperm Preparation: Husband's semen sample was collected by masturbation into a sterile semen container after 2-3 days of sexual abstinence. After liquefaction of sample at room temperature, quality of the semen was analyzed by World Health Organization (WHO) guideline and Kruger's strict criteria for sperm morphology [8].

The wash and spin method was used to prepare sperm for the IUI procedure. The specimen was mixed with bicarbonate based sperm wash medium (1:1) and centrifuged at 300-500 g for 10 minutes. The supernatant was discarded and the pellet was suspended in 1ml sperm wash medium and were kept at 37°C for 30 minutes in a 5% CO₂ based incubator. Upper two-third portion of suspension was collected in a sterile tube by Falcon 3 ml transfer pipette. The collected suspension was centrifuged at 300 g for 5 minutes and removed supernatant from the sterile tube. Finally, sperm pellet containing 0.35 ml sperm wash medium was kept in the humidified incubator for IUI. Post wash sperm concentration, motility and morphology was evaluated before IUI procedure.

Insemination Procedure: It was performed by using a soft flexible plastic catheter, while patient was in lithotomy position and sterile Cusco's self-retaining *bivalve* speculum was inserted into the vaginal canal. Post wash sperm filled IUI catheter was gently inserted into the cavity and slowly infused the sample. Patient had one hour bed rest in the procedure room after insemination. Each patient had taken progesterone suppository 400 mg per vaginally twice daily as a luteal phase support from the day of IUI procedure. Serum Beta HCG was done for every patient after two weeks of procedure to confirm pregnancy. Clinical pregnancy was determined by intra uterine gestational sac in the uterine cavity through trans-vaginal ultrasound.

Outcome Measures: Primary outcome was measured by pregnancy rate and secondary by live birth, pregnancy loss and multiple pregnancy rate. Pregnancy was confirmed by positive beta HCG test after a day of missed period. Expulsion of an alive baby from the mother irrespective of duration of the pregnancy is considered as a live birth. Pregnancy loss included abortion and ectopic pregnancy. More than one gestational sac with or without cardiac activity in the ultrasound report was considered as multiple pregnancy.

2.2 Data Analysis

Performed by using IBM SPSS software (version 26). Continuous variables were reported as the mean±standard deviation (SD) and student t test was used to compare between the continuous groups. Categorical variables were expressed as frequency and Chi-square test (χ^2) was performed to compare categorical variables.

3. RESULTS

Baseline characteristics of pregnant and non-pregnant groups are shown in Table 1. Both groups had similar basic characteristics. There were no significant difference in the mean characteristics of women age, body mass index (BMI), endometrium thickness, initial motile sperm of husband, and inseminated harvested sperm of husband. The mean duration of married life and number of mature follicles of pregnant group were significantly lower than non-pregnant group ($P<0.05$).

Fig. 1 shows the outcome IUI. A total 103 pregnancy were achieved from 518 IUI cycles of

426 participant couple. Overall pregnancy rate was 19.8%, where the live birth rate was 11.2% (58 out of 518). There were 49 babies born as a single and 9 babies were twin baby. One triplet pregnancy underwent abortion at second trimester of the pregnancy.

Overall, IUI procedure with more three follicle yield highest pregnancy than single or double follicle, $p<0.007$ (Table 2). Frequency of twin live birth as per pre ovulatory follicle: 2 out of 173 double follicle, 1 out of 69 triple follicle, 2 out of 39 four follicle and 1 out of 26 multiple follicle cases.

Cluster of follicle numbers in different age categories demonstrated that double follicle is predominant in each group. Multiple follicular response including three follicle, four follicle or multi follicle shows common trend of high proportion (Fig. 2). Fig. 3 shows the higher number of Beta HCG positive found in normal ovarian reserve than woman with low ovarian reserve as an outcome of IUI.

Table 1. Baseline characteristics of pregnant and non-pregnant group (n=426)

Trait	Pregnant	Non-Pregnant	P Value
	Mean±SD	Mean±SD	
Women age (yrs)	30.56±4.31	31.89±4.79	0.30
Married Life (yrs)	6.47±3.72	7.26±4.67	0.05
Body mass index of women	26.28±4.09	25.99±3.78	0.155
Number of mature follicle	2.77±1.26	2.44±1.03	0.00
Initial total motile sperm(M)	29.55±22.22	28.20±42.57	0.89
Inseminated Harvested sperm(M)	7.15±3.45	6.79±3.13	0.20
Endometrial Thickness	8.83±1.38	8.08±1.02	0.10

n: Total number of couple participated

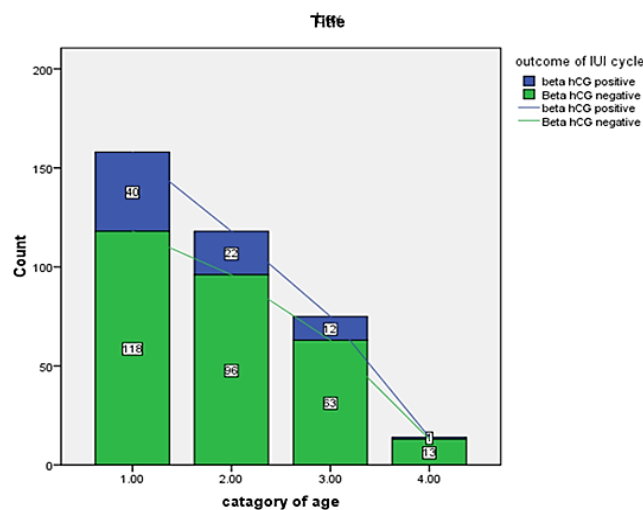


Fig. 1. Outcome of IUI according to age category

Table 2. Final follicular response after taking ovulation induction drugs (n=365)

Final follicular response after taking ovulation induction drug	Final status of pregnancy			Total	P value*
	Live birth	Abortion	Failed cycle		
Single follicle	6	5	47	58	0.007
Double follicles	15	10	148	173	
Tripple follicles	7	6	56	69	
Quadriple follicles	10	3	26	39	
Multiple follicles	5	6	15	26	
Total	43	30	292	365	
	11.8%	8.2%	80.0%	100.0%	

* Chi-square test was performed

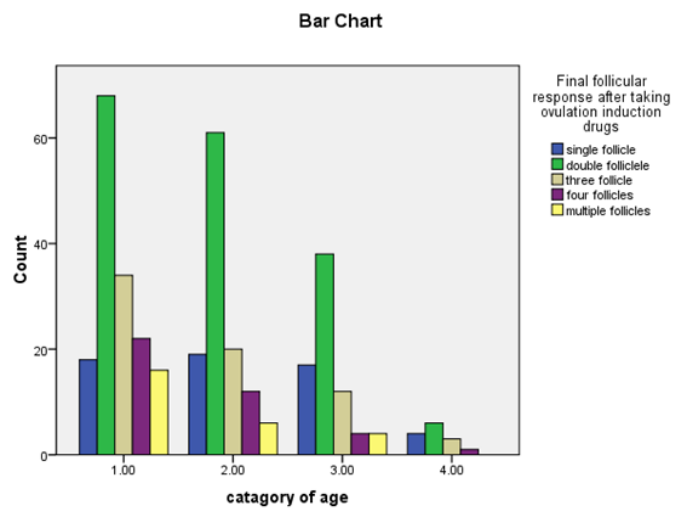


Fig. 2. Follicular response using induction drugs

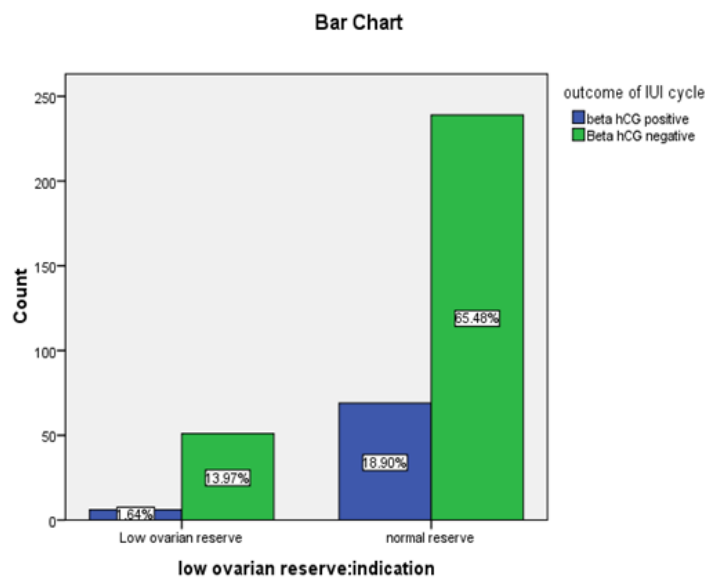


Fig. 3. Outcome of IUI cycles

4. DISCUSSION

The present research was conducted at Fertility Center of Evercare Hospital Dhaka, Bangladesh to determine prognostic factors of successful intrauterine insemination following controlled ovarian stimulation where a higher mean \pm SD was observed in respect of mean age of women, body mass index, initial total motile sperm and inseminated harvested sperm and endometrial thickness in non-pregnant women than pregnant that was not statistically significant. But a significant difference was observed in respect of number of mature follicle in pregnant than non-pregnant women by researcher [9]. So, optimum follicle number for successful IUI is pragmatic question because of cancellation of more than three follicles may proceed IVF or continuous treatment as an aggressive approach.

In the current research the overall pregnancy rate and live birth rate were 19.8% and 11.2% respectively and more than three follicles yield highest number of pregnancy than single or double follicle. While reporting cluster of follicle number in different age categories double follicle found predominant. Multiple follicular response including three follicle, four follicle or multi follicle shows common trend of high proportion. A higher number of Beta HCG positive found in normal ovarian reserve than woman with low ovarian reserve as an outcome of IUI. Although, we reported only one triplet (1/43) and 6 twins (6/43) in this study, Beta HCG positive cases (20.1%) gradually declined during gestational period and finally reached to 11.8% as live birth rate; even though our pregnancy rate and live birth rate were correspond to previous studies. An identical previous study also reported 8.6% twin pregnancy and no high order multiple pregnancy in 1,010 IUI cycles [6].

Particular attention on high order multiple pregnancy and live birth rate both were not correlated with multiple mature follicle demonstrated that multiple mature follicle associated with increased IUI pregnancy rate for unexplained infertility. This findings were supported by many investigators including a recent meta-analysis by van Ramste et al. who reported increased pregnancy rate with high order mature follicle compare to single mature follicle [10]. In this sense, previous retrospective study on different etiology have reported 0.6% risk of high order multiple pregnancy for multiple mature follicle [10]. A large retrospective study with gonadotropins by demonstrated 107 high

order pregnancy (5.4%) out of 1778 pregnancy from a single entre [11].

Though their study not particularly focused on unexplained infertility patient, they reported that young women (<32 years) were almost at 10 fold increased risk in same high estradiol (>862 pg/ml) and high follicle number than older women (>32 years).

Our study included only unexplained infertile women with older age (>32years), which could be probable reason for very low number of high order multiple pregnancy and also did not investigate the peak estradiol level on the day of HCG, only drawback in our study. A previous study showed 33.4 % pregnancy rate in a gonadotropin stimulated with IUI cycle. In this study we obtained only six twin pregnancy and one triplet pregnancy. A meta-analysis showed that risk of multiple pregnancy increased by 6%, 14% and 10% respectively after two, three and four mature follicle IUI A study demonstrated that sperm motility impact and success of IUI [10]. A considerable variation of time for both ovulation and sperm capacitation also in favor of low pregnancy rate [12].

5. CONCLUSION

Here the key pregnancy determining factors for IUI were women's age, duration of infertility and types of infertility. Women child bearing capacity significantly decreases after the age 35 years. Ultrasound based factors that impacts on pregnancy outcome were number of preovulatory follicle and endometrium thickness. Among indications of IUI, factors that influenced pregnancy were anovulation, low ovarian reserve and single tubal patency. We did not find significant correlation of IUI pregnancy with total motile sperm and harvested inseminated sperm.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was taken from Ethical Committee of Bangladesh Fertility Hospital Ltd. (Memo No- BFHL/EC/2023/03). All methods were carried out in accordance with relevant guidelines and regulations as per ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barrera N, Omolaoye TS, Du Plessis SS. A contemporary view on global fertility, infertility, and assisted reproductive techniques. In *Fertility, Pregnancy, and Wellness*. Elsevier. 2022;93-120.
2. van Rumste MM, den Hartog JE, Dumoulin JC, Evers JL, Land JA. Is controlled ovarian stimulation in intrauterine insemination an acceptable therapy in couples with unexplained non-conception in the perspective of multiple pregnancies? *Human Reproduction*. 2006;21(3):701-704.
3. Bergh C, Kamath MS, Wang R, Lensen S. Strategies to reduce multiple pregnancies during medically assisted reproduction. *Fertility and Sterility*. 2020;114(4):673-679.
4. Land JA, Evers JL. Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. *Human Reproduction*. 2003;18(2):455-457.
5. van Wely M, Twisk M, Mol BW, Van der Veen F. Is twin pregnancy necessarily an adverse outcome of assisted reproductive technologies? *Human Reproduction*. 2006; 21(11):2736-2738.
6. Steiner N, Al Shatti M, Frank R, Rotshenker-Olshinka K, Ruitter-Ligeti J, Dahan MH. Relationship between Number of Mature Follicles and Pregnancy Rates in IUI Cycles in Women 38 to 43 Years Old. *Clinical and Experimental Obstetrics & Gynecology*. 2023;50(2):23.
7. Ibérico G, Vioque J, Ariza N, Lozano JM, Roca M, Llácer J, Bernabeu R. Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. *Fertil Steril*. 2004;81(5):1308-1313.
8. World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th ed. Geneva: WHO Press; 2010.
9. Carré J, Gatimel N, Moreau J, Parinaud J, Léandri R. Does air pollution play a role in infertility?: a systematic review. *Environ Health*. 2017;16(1):1-16.
10. Aboulghar M, Manasour R, Serour G, Abdrazek A, Amin Y, Rhodes C. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of unexplained infertility should be limited to a maximum of three trials. *Fertil Steril*. 2001;75(1):88-91.
11. van Rumste MM, Custers IM, van der Veen F, van Wely M, Evers J, Mol BWJ. The influence of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: a meta-analysis. *Hum Reprod Update*. 2008; 14(6):563-570.
12. Nandi A, Bhide P, Hooper R, Gudi A, Shah A, Khan K, Homburg R. Intrauterine insemination with gonadotropin stimulation or in vitro fertilization for the treatment of unexplained subfertility: a randomized controlled trial. *Fertil Steril*. 2017;107(6): 1329-1335.