Original Article

Single versus double intrauterine insemination—in artificial insemination donor stimulated cycles—impact on the clinical pregnancy rate: A randomized trial

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Abstract

Introduction: Male factor infertility affects almost 30–50% of infertile couples worldwide. The use of donor sperm intrauterine insemination (IUI) is an option for couples with absolute azoospermia, severe male factor infertility, sexual dysfunction and unaffordability for *in-vitro* fertilization. The data on the utility of double IUI using donor sperm are limited due to the lack of randomized controlled trial's and conflicting conclusions. The objective of this study was to evaluate the utility of frozen double donor sperm IUI to improve clinical pregnancy rate.

Materials and Methods: A total of 200 participants that fulfilled the inclusion criteria were enrolled for the study following randomization after preliminary work up. Random distribution was made using sequentially numbered envelope method in both groups (single and double IUI).

Results: In this dataset comparing single and double donor sperm IUI, there was no significant difference in clinical pregnancy rates. The clinical pregnancy of 11 participants (11%) (out of 100 participants) in single IUI group and 13 participants (13%) (out of 100 participants) in double IUI group was seen.

Conclusion: However, clinical pregnancy rate was statistically significant with gonadotrophins in double IUI group in our study.

Keywords: clinical pregnancy rate, double IUI, male infertility, single IUI

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INTRODUCTION

Male factor infertility affects almost 30–50% of infertile couples worldwide, with most infertile men experiencing low sperm density or other semen abnormalities without the presence of any specific underlying cause. In these men with idiopathic oligospermia, asthenospermia or teratospermia, although medical treatment may improve semen quality parameters, it is unclear whether such a clinical practice may indeed improve fertility in general. Taking into account that the possibility of spontaneous pregnancy is 2% without any therapy, assisted

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reproduction is the next logical step to increase the possibility of pregnancy.

Until recently, intrauterine insemination (IUI) was considered the standard method of treatment in the participants of unexplained and male factor subfertility. Unexplained infertility is the labeled as the cause when no cause can be attributed for infertility despite fully investigating both the partners. It accounts for 30% of causes of infertility. Several studies supported IUI as having the same likelihood of successful pregnancy as *in-vitro* fertilization (IVF) while being more cost-effective, with the cost of IUI per pregnancy remaining four to seven times lower than the cost of IVF. Nonetheless, despite the availability, and ease of performing IUI, other studies support the use of IVF as a first line of therapy based on their findings of higher success rates, shorter times to pregnancy and a trend towards fewer multiple pregnancies.

The use of donor sperm IUI is an option for couples with absolute azoospermia, severe male factor infertility, sexual dysfunction and unaffordability for IVF. For couples with severe male factor infertility, donor sperm IUI provides substantial cost benefit compared to microdissection testicular sperm extraction and intracytoplasmic sperm injection.[1]

There are many factors such as causes of infertility, age, number of dominant follicles, the history of pelvic diseases, endometrial thickness and duration of infertility that can have a effect on the success rate of IUI.[2] IUI has demonstrated higher pregnancy rates of approximately15–20% when ovulation induction performed with gonadotrophin as compared to 10–15% with clomiphene citrate (CC).

Moreover, it seems that considerable changes made in the protocol of IUI including timing and the frequency can enhance the success rate. Double IUI is a method with increased frequency and change in timing versus single IUI. To further increase the total concentration of sperm delivered and the window of sperm exposure to the oocyte, performing IUIs on two consecutive days (double IUI) has been proposed to increase pregnancy rates.[3]

The data on the utility of double IUI using donor sperm are limited due to the lack of randomized controlled trial's (RCT) and conflicting conclusions. The objective of this study was to evaluate the utility of frozen double donor sperm IUI to improve clinical pregnancy rate.

MATERIALS AND METHODS

Study design: A RCT.

Place of study: Department of Reproductive Medicine & Medical Genetics (Obstetrics & Gynaecology), Mahatma Gandhi Medical College & Hospital, Jaipur, Rajasthan.

Duration of study: 17 months (April 2016 to October 2017).

Inclusion criteria:

(1) Case of absolute azoospermia, unexplained infertility (when no attributable cause can be determined for infertility despite fully investigating the couple), severe oligoasthenospermia not affordable for IVF.
(2) Women of age group 25–30 years of age.
(3) Normal uterine cavity.
(4) At least one patent tube.

Exclusion criteria:

(1) Baseline serum follicle stimulating hormone (FSH) > 12 IU and serum luteinizing hormone (LH) > 10 pg/ml.
(2) Genital tuberculosis.
(3) Polycystic ovary disease.
(4) Severe endometriosis (gr3 and 4).
(5) Chronic pelvic inflammatory disease.
(6) Tubal block.
(7) Uterine and cervical anomalies.
(8) Any history of hypersensitivity to the study drug.

A total of 200 participants that fulfilled the inclusion criteria were enrolled for the study following randomization after preliminary work up. Random distribution was made before ovulation induction using sequentially numbered opaque envelope method (SNOSE method) in both groups (single and double IUI). A total of 104 participants received clomiphene ($n = 52$ in each group), 56 participants underwent stimulation with clomiphene followed by gonadotrophin ($n = 28$ in each group) and 40 participants received stimulation with gonadotrophin ($n = 20$ in each group) [Figure 1]. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, United States).

Preliminary work up included demographic profile: age, occupation, education, socioeconomic status. History of infertility: primary or secondary infertility, duration of infertility, history of previous treatment. Menstrual history, obstetric history, sexual history, past history—H/O hypertension, diabetes, thyroid ds, renal ds. sexually transmitted disease (STD) was also noted.

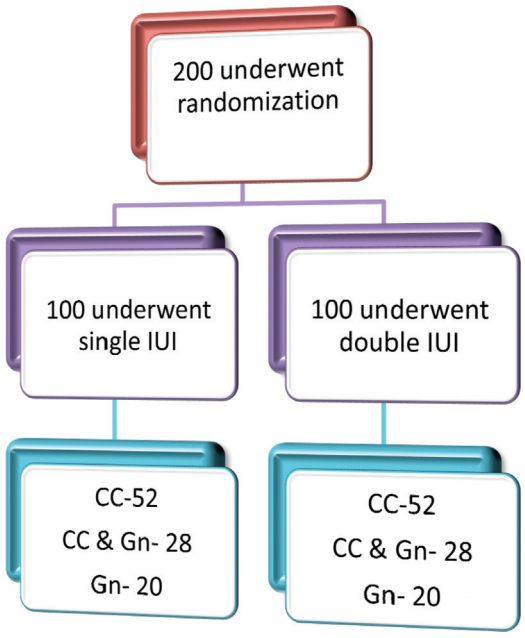

Figure 1: Flow chart depicting randomization

General physical examination included height, weight, body mass index (BMI), thyroid enlargement, signs of androgen excess—hirsuitism, acne, virilisation. A detailed gynecological examination was performed which included—per speculum examination to look for discharge, secretion, cervical or vaginal abnormality, and per vaginal examination to note size, shape, consistency, position of uterus and cervix, mass, tenderness or nodularity in adenexa, to rule out any infection or disorder.

Each time in transvaginal sonography (TVS), following details were taken—number of follicle, follicular diameter, endometrial thickness and follicular rupture confirmed via reduction in size of follicle, irregular shape of follicle, increased echogenicity inside follicle and free fluid in pouch of Douglas.

Patients had a stimulated cycle with clomiphene citrate 50/100 mg from day 3 to 7 of menstrual cycle or human menopausal gonadotrophin (HMG) 75–150 IU from day 3 of menstrual cycle till dominant follicle reaches 18 mm or clomiphene 50/100 mg from day 3 to day 7 of menstrual cycle with gonadotrophin added from day 7 of menstrual cycle depending on age, BMI and baseline FSH of patient. The cycles were monitored with transvaginal ultrasound and serum estradiol measurements. Endometrial vascularity was also noted in transvaginal scan. Ovulation trigger was planned when the leading follicle was ≥18 mm. Ten thousand units of intramuscular human chorionic gonadotrophin (hCG) was administered in the evening around 6 PM. Single IUI was performed 38–40 h after beta hCG injection. Donor double IUI was performed on two mornings following ovulation induction (when dominant follicle 18–20 mm was found), i.e., 18–20 and 38–40 h after hCG trigger. A Soft Pass insemination catheter was used for the IUI. Participants were asked to remain supine for 20 min after the insemination procedure. Progesterone support—natural micronized progesterone 200 mcg HS vaginally was given for 15 days following IUI. Serum hCG levels were assessed 2 weeks after second IUI and ultrasonographic confirmation of pregnancy was obtained in all pregnant patients 2 weeks after serum hCG. Clinical pregnancy was defined as the presence of an intrauterine gestation with fetal cardiac activity.

Donor semen was obtained from sperm bank well recognized under all rules and regulations laid down by government of India. The frozen semen was prepared by the conventional swim-up technique in both groups. The semen samples were thawed at room temperature for 10–15 min. After liquefaction the sample was diluted with Earl's balanced salt solution media in the ratio of 3:2 and centrifuged for 10 min at 1800 rotation per minute. This procedure was repeated twice. The supernatant was discarded and the pellet was resuspended in 1 ml of IVF 30 culture media and centrifuged for 3 min at 1800 rotation per minute and the supernatant was removed again. The final pellet was resuspended in 1 ml of culture media without allowing the sperm pellet and media to mix and incubated at 37°C in 5% CO_2 for 30 min. Finally 0.3 ml of the top layer containing highly motile fraction of spermatozoa was aspirated gently in a 1 ml syringe and sperm concentration and motility was ascertained once again.

The sperm parameters before and after preparation were compared. The pre- and postwash semen characteristics that were reported included sperm concentration, percentage of sperm motility, viability and normal morphology.

Morphology testing involved examination of structure of sperm using strict Krugerberg's criteria. Lower normal limit should account for 4% of total [World Health Organization (WHO) criteria].

Ethical clearance and written informed consent of all the patients were obtained before enrolling them in study.

RESULTS

Data analysis was performed using the SPSS software [Table 1 and Figure 2].

In our study, the mean age of participants in single IUI was 29.4 years compared with double IUI, where it was 30.8 years, which was not statistically significant [Table 2 and Figure 3].

Table 1: Demographics parameters of patients

	Single IUI (n = 100)	Double IUI (n = 100)	*P* value
Age	29.5	30.8	>0.05
BMI	29	27.7	>0.05
Duration of infertility	6	5	>0.05
Type of infertility			
Primary	74	76	>0.05
Secondary	36	34	>0.05

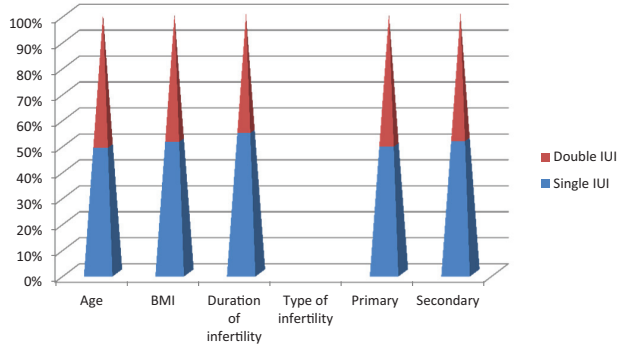

Figure 2: Demographics parameters of patients

Table 2: Mean semen parameters (postwash) on day of single and double IUI

	Single IUI	Double IUI	*P* value
Total sperm count (10^6/million)	77	75	>0.05
Motility (progressive)	58	59	>0.05
Viability	79	82	>0.05
Morphology (%) (strict Kruger Tygerberg criteria)	6	7	>0.05

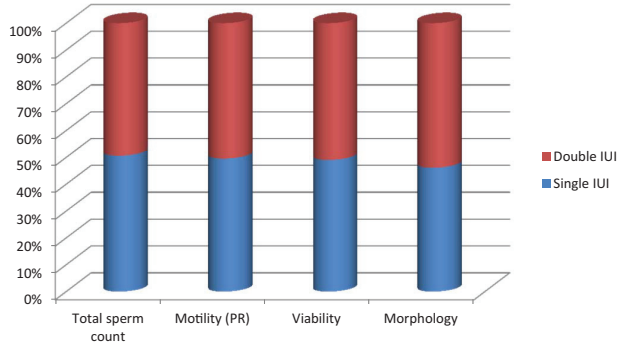

Figure 3: Mean Semen Parameters (Post Wash) on day of single and double IUI

Taking into account, the semen parameters (postwash), total count, motility, viability and morphology—no statistically significant difference was found in single and double IUI group [Table 3 and Figure 4].

Among ultrasound and hormonal parameters S. FSH level, E2 level, number of dominant follicle and endometrial thickness[5,6] no statistically significant difference was found [Tables 4 and 5].

In our study, clinical pregnancy of 11 participants (11%) (out of 100 participants) was found in single IUI group and 13 participants (13%) (out of 100 participants) was found in double IUI group. However there was no statistically significant difference in clinical pregnancy rate in both the groups [Figure 5].

Table 3: Hormonal and ultrasound parameters

	Single IUI (n = 100)	Double IUI (n = 100)	*P* value
Baseline FSH	6	5	>0.05
Antral follicle count	8	7	>0.05
No. of dominant follicle	1.6	1.8	>0.05
Endometrial thickness (mm)	7.6	7.8	>0.05
Serum E2 level	380	410	>0.05
Endometrial vascularity	II	II	>0.05

FSH, follicle stimulating hormone.

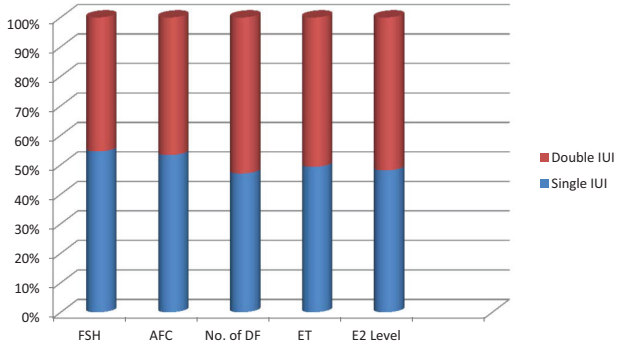

Figure 4: Hormonal & Ultrasound Parameters

Table 4: Outcome measures studied

	Single IUI (n = 100)	Double IUI (n = 100)	*P* value
Beta hCG positive	12	14	>0.05
Missed abortion	1	1	>0.05
Clinical pregnancy rate	11	13	>0.05

hCG, human chorionic gonadotrophin.

Table 5: Clinical pregnancy rate with different controlled ovarian hyperstimulation (COH) protocol

	Single IUI (n = 100)	Double IUI (n = 100)	*P* value
Clomiphene citrate (n = 104)	5	3	>0.05
Clomiphene citrate with HMG (n = 56)	3	4	>0.05
HMG (n = 40)	3	6	0.04 (<0.05)

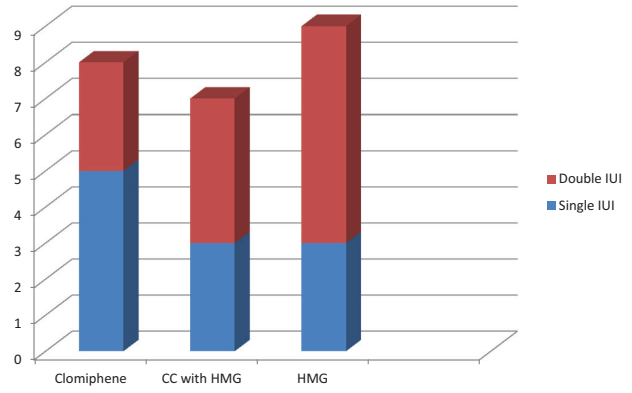

Figure 5: Comparison of clinical pregnancy rate in single & double IUI

DISCUSSION

A total of 200 participants were included in our study after randomization via sequentially numbered opaque envelops, 100 in each group. In our study mean age of participants in single IUI was 29.4 years compared with double IUI it was 30.8 years which was not statistically significant.

Mean BMI of patients in single IUI group was 29 kg/m^2 as opposed to 27.7 kg/m^2 in double IUI group which was not significant statistically. Mean duration of infertility was 6 years in single IUI and 5 years in double IUI group, which was statistically significant.

Taking into account the semen parameters (postwash), total count, motility, viability and morphology—no statistically significant difference was found in single and double IUI groups, which is further supported by studies conducted by Ranson *et al.* [7] and Johnston *et al.* [8] showing no significant difference.[4] Semen preparation was found to be cost effective in our study.

Among ultrasound and hormonal parameters, S. FSH level, E2 level, number of dominant follicle and endometrial thickness.[5,6] No statistically significant difference was found supported by study by Bagis *et al.* [9] and Kaser Daniel *et al.* [10]

In this dataset, comparing single and double donor sperm IUI, there was no significant difference in clinical pregnancy rates. Our data with clinical pregnancy of 11 participants (11%) (out of 100 participants) in single IUI group and 13 participants (13%) (out of 100 participants) in double IUI group is consistent with two other studies by Khalifa *et al.*[16] and Chavkin *et al.*[20] in showing no benefit with the addition of double IUI for donor sperm cycles. These results are in contrast with the findings from Matilsky *et al.*[17] which demonstrated the benefit of double insemination with a clinical pregnancy rate of 17.9% per cycle versus 5% per cycle with single insemination. In a study by Pathak *et al.*,[18] better pregnancy rate has been achieved in double than single IUI in both groups, that is, 16.17% versus 20.33% in infertility up to 5 years and 12.90% versus 17.14% in infertility for 6–10 years group.[11]

Although data from a meta-analysis (Cochrane 2003) have shown that double IUI does not significantly improve pregnancy rates among women with unexplained infertility, such an approach may have a markedly different outcome among couples with male factor infertility. The rationale for the use of double IUI is that the cumulative number of motile spermatozoa inseminated in double IUI cycles is higher than that in single IUI cycles.[12] The increase in IUI frequency results in more motile spermatozoa being delivered to the site of fertilization in each treatment cycle, and this may increase pregnancy rates. Furthermore, with double IUI, the time of spermatozoa presence is longer, which may contribute to the increase in the success rate of IUI per cycle. The time of insemination is very important for the success of IUI,[13] because follicle rupture may occur over a long time interval (24–48 h). Double IUI could be helpful in male factor with oligoasthenospermia but not in donor participants, as semen sample is ideal in donor IUI.

Clinical pregnancy rate is the primary outcome studied followed by clinical pregnancy rate resulting from different stimulation protocol used being the secondary outcome studied. However, there was no statistically significant difference in clinical pregnancy rate in both the groups, but with gonadotrophin stimulation protocol clinical pregnancy rate was statistically significant in double IUI (6 out of 20 in double IUI versus 3 out of 20 in single IUI) the reason remains undefined. Improved oocyte and endometrial quality by gonadotrophin can be one of the reasons however because of small sample size we cannot comment in a defined way.

Clomiphene citrate was considered as first line of drug for ovulation induction.[14] However, in our study, we have achieved better pregnancy rate with gonadotrohin. Clomiphene citrate antiestrogenic action does result in poor endometrial growth and change in cervical mucus. Clomiphene citrate negatively affects endometrial thickness, subendometrial blood flow, oocyte quality, embryo development, and hence ultimately, the pregnancy rates are compromised. In the prospective randomized trial, Chavkin *et al.*[20] showed that the endometrium is significantly thinner in the group

where CC was given. Addition of gonadotropins also elicited favorable response on the endometrium but not reaching statistical significance on the day of trigger. This finding is consistent in our study too (7.6 in single IUI and 7.8 in double IUI group).

Although initial reports regarding double IUI show promise for couples with male factor infertility, the current available evidence does not have the power or the consistency to support such a shift in clinical practice.[15,17] In addition, we should consider the fact that double IUI increases both the financial cost and burden on the health provider and the couple compared with single IUI.[15,16,19] Ultimately, large sample size trials are needed to address this topic.

CONCLUSION

In conclusion, this study did not demonstrate a benefit to the routine use of double IUI in donor sperm cycles. Adding a second IUI increases the cost. Given the current lack of evidence supporting a benefit of double over single donor IUI, we believe that routine clinical use of double donor IUI is not justified. However clinical pregnancy rate was statistically significant with gonadotrophins in double IUI group in our study; the reason behind is that it needs further evaluation supported by RCT's.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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