Original Article

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Efficacy of Double versus Single Homologous Intrauterine Insemination in Mild Male Factor Infertility and Pregnancy Outcome: A Randomised Controlled Trial

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Intrauterine Insemination (IUI) is a cost-effective, minimally invasive, and widely accepted Assisted Reproductive Technology (ART) procedure used to treat infertility. Various factors contribute to infertility, which in turn affect the success rate of 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 enhance pregnancy rates, especially in cases of oligospermia. Double IUI aims to address the challenge of synchronising ovulation and insemination.

Aim: To assess the efficacy of double IUI compared to single insemination in cases of mild male factor infertility.

Materials and Methods: A randomised clinical study was conducted in the Department of Obstetrics and Gynaecology (OBG), Shri Dharmasthala Manjunatheshwara College of Medical Sciences and Hospital Fertility Centre, Sattur, Dharwad, Karnataka, India from July 2021 to January 2022 on 200 patients. All 200 patients underwent ovulation induction with a sequential regimen using Letrozole or Clomiphene citrate, alongside highly purified Human Menopausal Gonadotropin (75 IU). All women underwent follicular monitoring and received an ovulation trigger. The first group of patients underwent a single IUI 36 hours after the Human Chorionic Gonadotropin (hCG) injection. Patients in the second group, who underwent double IUI, received the first IUI at 24 hours and the second IUI 48 hours after the hCG injection. Serum beta hCG was measured 15 days after the IUI to confirm the occurrence of pregnancy.

Results: There were a total of 42 pregnancies (21%). The pregnancy rate for the single IUI group was 20%, while for the double IUI group, it was 22%. There was no significant difference between the single IUI and double IUI groups regarding positive outcomes (Chi-square=0.1210, p=0.7280).

Conclusion: Double IUI increases the total concentration and quality of sperm delivered and extends the window of sperm exposure to the oocyte. This additional procedure may translate to increased pregnancy rates. However, in present study, no significant difference was noted in the achieved pregnancy rates. Higher-quality studies with larger populations are required to formulate a hypothesis.

Keywords: Assisted reproductive technique, Female infertility, Homologous, Insemination, Male infertility, Oligospermia, Ovulation induction, Pregnancy rates

INTRODUCTION

Intrauterine Insemination (IUI) is a common ART approach for couples experiencing a wide range of infertility issues. It is the preferred treatment modality for patients before opting for In-vitro Fertilisation (IVF) or Intracytoplasmic Sperm Injection (ICSI) due to its cost-effectiveness, minimally invasive nature, and simplicity of execution.

There are many factors contributing to infertility, such as age, ovarian reserve, history of pelvic diseases, endometrial thickness, and duration of infertility, which determine the success rate of IUI [1-4]. 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 enhance pregnancy rates, especially in cases of oligospermia. IUI during stimulated cycles appears to be advantageous in achieving conception [4,5]. Double IUI has been suggested to address the challenge of synchronising ovulation and insemination.

Considering that the possibility of spontaneous conception is only 2%, ART is the next logical step to increase the chances of pregnancy [6]. IUI has various determinants of success. Among these, sperm morphology, the method of sperm preparation, the cause of infertility, the ovulation induction regimen used, the agent for ovulation triggering, age, duration of infertility, number of follicles, endometrial thickness, and history of pelvic infections are important. Additionally, the time interval between ovulation induction and IUI, as well as the time interval between sperm preparation and IUI, are clinically relevant factors [7,8].

If sperm is available at the right time for insemination, fertilisation is likely to occur. The idea of multiple inseminations during a cycle could help capture the precise moment for fertilisation and increase pregnancy rates [9]. The rationale for double insemination is to provide a longer fertilisation period, as follicle rupture may occur over a wide interval (approximately 22-47 hours) after Human Chorionic Gonadotropin (hCG) administration in ovarian hyperstimulation/IUI cycles.

The IUI is typically performed with a single insemination of prepared semen about 24 to 36 hours after hormone administration for final ovulation triggering. The double insemination technique has been proposed to maximise oocyte exposure to sperm, ultimately aiming to improve pregnancy rates after IUI. Because the timing of ovulation following the triggering is believed to vary with the ovarian stimulation protocol and a woman's individual characteristics [10], double IUI has been suggested to address the issue of synchronising ovulation and insemination [11].

While studies have been conducted on double IUI in cases of female factor infertility, similar studies in mild male factor infertility are lacking [12,13]. Therefore, present study was conducted to assess the efficacy of double IUI compared to single insemination in couples with mild male factor infertility.

MATERIALS AND METHODS

A randomised controlled study was conducted in the Department of Obstetrics and Gynaecology (OBG), Shri Dharmasthala Manjunatheshwara College of Medical Sciences and Hospital Fertility Centre, Sattur, Dharwad, Karnataka, India conducted from July 2021 to January 2022. Institutional Ethical Committee permission was obtained (SDMIEC/2021/53).

Sample size calculation: Hypothesis testing for two means (equal variances) was utilised. Based on the pilot study with washed sperm count (mill/mL) after post-test:

Standard deviation in the first group: S1=31.29

Standard deviation in the second group: S2=22.86

Mean difference between groups: 11.69

Effect size=0.431763619575254

Alpha Error (%)=5

Power (%)=85

Sided=2

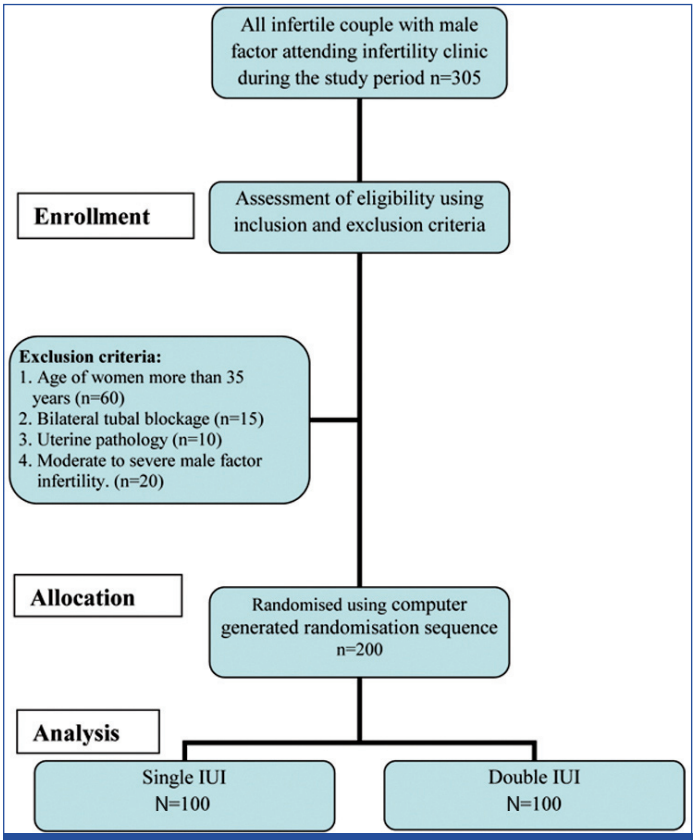
Number needed (n)=100 in each group

Inclusion criteria: Age of the women less than 35 years and normal uterine cavity, atleast one patent tube and mild male factor infertility were included in the study.

Exclusion criteria: Age of women more than 35 years with bilateral tubal blockage and uterine pathology, moderate to severe male factor infertility were excluded from the study.

Study Procedure

A total of 2,727 infertile patients attended the fertility center Outpatient Department (OPD) from July 2021 to January 2022. After evaluation, 305 couples were identified with male factor infertility. Of these patients, 200 who met the inclusion and exclusion criteria were enrolled in the study [Table/Fig-1]. All 200 patients allocated for the study underwent ovulation induction with a sequential regimen using Letrozole or Clomiphene citrate for five days. If the dominant follicle was not recruited by day 8, highly purified human menopausal gonadotropin (75 IU) was added for one or two doses. All women underwent transvaginal sonography for follicular monitoring.



[Table/Fig-1]: Consodilated Standards of Reporting Trails (CONSORT) flow diagram depicting the study participants.

When the leading follicle reached 18-20 mm, ovulation was triggered using hCG 10,000 units as a single intramuscular dose. Randomisation of patients into two groups was carried out using simple randomisation with a computer-generated randomisation sequence.

In the first group, patients underwent single IUI, which was performed 36 hours after the hCG injection. Patients in the second group, who underwent double IUI, received the first IUI 24 hours after hCG and the second IUI 48 hours after the hCG injection. All patients underwent transvaginal sonography to confirm ovulation.

Semen samples were prepared using the double density gradient method. Shorr stain was used for morphology testing, and sperm viability was assessed after staining with Haematoxylin and Eosin (H&E). The IUI was performed within one and a half hours of sample collection. All patients received luteal phase support. Serum beta hCG was measured 15 days after the IUI to confirm the occurrence of pregnancy.

STATISTICAL ANALYSIS

Data analysis was conducted using Statistical Package for Social Sciences (SPSS) software version 20.0. Descriptive analysis was performed using percentages for categorical variables and the median with Interquartile Range (IQR) for continuous variables. The Chi-square test was used for categorical variables, while the Student's t-test was employed for continuous variables.

RESULTS

A total of 200 patients were recruited for the study. Total 100 patients underwent single IUI 36 hours after the hCG trigger, while another hundred patients underwent double IUI, 24 and 48 hours after the hCG trigger. In the single IUI group, there were 25 cases (25%) in the 21-25 year age range, 41 cases (41%) in the 26-30 year range, and 34 cases (34%) in the 31-35 year range. In the double IUI group, there were 14 cases (14%) in the 21-25 year age range, 56 cases (56%) in the 26-30 year range, and 30 cases (30%) in the 31-35 year range [Table/Fig-2].

Age group	Single IUI	Double IUI	Total n (%)	χ^2	p-value
21-25 y	25	14	39 (19.50)	5.6720	0.0590
26-30 y	41	56	97 (48.50)		
31-35 y	34	30	64 (32)		
Age (mean±SD)	28.63±3.57	28.74±3.37	28.69±3.46		
Total	100	100	200 (100)		

[Table/Fig-2]: Comparison of single IUI and double IUI according to age.

In the single IUI group, 72 patients (72%) had primary infertility and 28 had secondary infertility, while in the double IUI group, 83 patients (83%) had primary infertility and 17 patients (17%) had secondary infertility [Table/Fig-3].

PI/SI	Single IUI	Double IUI	Total n (%)	χ^2	p-value
PI	72	83	155 (77.50)	3.4700	0.0630
SI	28	17	45 (22.50)		
Total	100	100	200 (100)		

[Table/Fig-3]: Comparison of single IUI and double IUI according to Primary Infertility/Secondary Infertility.

Based on the weight of the patients, there was no statistically significant difference observed between the two groups [Table/Fig-4].

In terms of female factors, the majority of cases in the single IUI group were due to PCOD (55 cases, 55%), followed by endometriosis (11 cases, 11%) and normal findings (11 cases, 11%). In the double IUI

Obesity	Single IUI	Double IUI	Total n (%)	χ^2	p-value
Under weight	5	4	9 (4.50)	2.7240	0.2560
Normal	28	39	67 (33.50)		
Overweight	67	57	124 (62)		
Mean±SD	26.15±4.33	25.14±4.01	25.64±4.19		
Total	100	100	200 (100)		

[Table/Fig-4]: Comparison of single IUI and double IUI according to obesity.

group, the majority of cases were also due to Poly Cystic Ovarian Disease (PCOD) (49 cases, 49%) and normal findings (21 cases, 21%) [Table/Fig-5].

Female factor	Single IUI	Double IUI	Total n (%)
Normal	11	21	32 (16)
Ashermans syndrome	1	1	2 (1)
BOH	1	0	1 (0.50)
BOR	4	16	20 (10)
DM	2	0	2 (1)
Endometriosis	11	6	17 (8.50)
Fibroid uterus	0	2	2 (1)
Left fimbrial block	1	0	1 (0.50)
Mild endometriosis	0	1	1 (0.50)
Minimal endometriosis	0	1	1 (0.50)
Obesity	1	0	1 (0.50)
PCOD	55	49	104 (52)
PID	1	2	3 (1.50)
RPL	2	0	2 (1)
Simple ovarian cyst	2	0	2 (1)
Subject: septate uterus and simple cyst	1	0	1 (0.50)
TB	0	1	1 (0.50)
Unexplained	7	0	7 (3.50)

[Table/Fig-5]: Comparison of single IUI and double IUI according to female factor.
BOH: Bad obstetric history; BOR: Borderline ovarian reserve; DM: Diabetes mellitus; PCOD: Polycystic ovarian disease; PID: Pelvic inflammatory disease; RPL: Recurrent pregnancy loss; TB: Tuberculosis

No statistical difference was observed based on male factors between the two groups [Table/Fig-6].

Male factor	Single IUI	Double IUI	Total n (%)	χ^2	p-value
Normospermia	92	96	188 (94)	1.4180	0.2340
Oligospermia	8	4	12 (6)		
Total	100	100	200 (100)		

[Table/Fig-6]: Comparison of single IUI and double IUI according to male factor.
No statistical difference was found between single IUI and double IUI based on conception

The pregnancy rate for the single IUI group was 20 patients (20%), while for the double IUI group, it was 22 patients (22%) [Table/Fig-7].

Results	Single IUI	Double IUI	Total n (%)	χ^2	p-value
Negative	80	78	158 (79)	0.1210	0.7280
Positive	20	22	42 (21)		
Total	100	100	200 (100)		

[Table/Fig-7]: Comparison of single IUI and double IUI according to pregnancy rates.

The mean sperm count in pre- and post-wash semen samples was comparable in both the single and double IUI groups [Table/Fig-8]. A statistical difference was found in pre-wash and post-wash samples for both the single IUI and double IUI groups, with a p-value of 0.0001 [Table/Fig-9].

Sperm count at	Single IUI Mean±SD	Double IUI Mean±SD	t-value	p-value
Pre-wash	60.66±43.11	60.27±36.97	0.0691	0.9450
Post-wash	30.44±19.59	31.32±17.64	-0.3319	0.7403
Difference	30.22±37.21	28.96±28.86	0.2692	0.7881

[Table/Fig-8]: Comparison of single IUI and double IUI with mean pre and post-wash SP count (mill/mL) by Independent t-test.
SD: Standard deviation

Groups	Treatment	Mean±SD	Mean diff.	Std. Dv. diff.	t-value	p-value
Single IUI	Pre-wash	60.66±43.11	30.22	37.21	8.1222	0.0001*
	Post-wash	30.44±19.59				
Double IUI	Pre-wash	60.27±36.97	28.96	28.86	10.0319	0.0001*
	Post-wash	31.32±17.64				

[Table/Fig-9]: Comparison of pre and postwash SP count (mill/mL) in single IUI and double IUI by Independent t-test.
*p<0.05

In sperm analysis, there was a significant difference in progressive motility after post-wash, with a p-value of 0.0028. Additionally, significant differences were observed in non progressive motility between pre-wash and post-wash samples, with p-values of 0.0451 and 0.0010, respectively [Table/Fig-10].

Motility at	Times	Single IUI Mean±SD	Double IUI Mean±SD	t-value	p-value
Progressive	Pre-wash	52.04±14.93	48.37±14.63	1.7581	0.0803
	Post-wash	91.03±8.40	86.32±13.09	3.0277	0.0028*
	Difference	38.99±14.73	37.96±16.96	0.4608	0.6454
Non progressive	Pre-wash	15.45±6.62	17.35±6.67	-2.0161	0.0451*
	Post-wash	4.66±4.71	8.71±11.20	-3.3285	0.0010*
	Difference	10.79±7.46	8.64±12.66	1.4633	0.1450
Total	Pre-wash	67.71±11.61	64.95±12.83	1.5948	0.1124
	Post-wash	95.09±7.64	94.65±5.05	0.4805	0.6314
	Difference	27.38±12.94	29.70±12.51	-1.2893	0.1988

[Table/Fig-10]: Comparison of Single IUI and Double IUI with mean pre and post-wash Progressive, pre and post-wash non progressive and pre and post-wash total motility % scores by Independent t-test.
*p<0.05

A significant difference was also seen in total motility (both progressive and non progressive) in both groups, with a p-value of 0.0001 [Table/Fig-11]. The morphological parameters of the sperm, specifically the percentage of normal sperm, were comparable between both groups [Table/Fig-12].

DISCUSSION

Post-wash semen parameters showed significant improvement in sperm count and motility, with a p-value of 0.0001. A total of 42 pregnancies were recorded, resulting in a positivity rate of 21%. The pregnancy rate for the single IUI group was 20%, while for the double IUI group, it was 22%.

The IUI is preferred over IVF/ICSI because it is cost-effective, less invasive, and a simpler procedure. Since its inception, several improvements have been implemented to increase the success of IUI, including pre-IUI semen preparation techniques to enhance semen parameters, timing of IUI with respect to ovulation [1,4], use of stimulated cycles during IUI [1,2,5,8], different sites of insemination [14], slow-release insemination [15], and multiple inseminations within the same cycle [1,9,16,17].

The success rate for IUI in present study was 21%, which is similar to a study conducted by Yumusak OH et al., who reported pregnancy rates of 22.9% and 26.9% in patients with PCOS and unexplained infertility, respectively [2]. According to Bahadur G and Homburg R,

Variable	Groups	Treatment	Mean±SD	Mean difference	SD difference	t-value	p-value
Progressive motility	Single IUI	Pre-wash	52.04±14.93	-38.99	14.73	-26.4780	0.0001*
		Post-wash	91.03±8.40				
	Double IUI	Pre-wash	48.37±14.63	-37.96	16.96	-22.3827	0.0001*
		Post-wash	86.32±13.09				
Non progressive motility	Single IUI	Pre-wash	15.45±6.62	10.79	7.46	14.4650	0.0001*
		Post-wash	4.66±4.71				
	Double IUI	Pre-wash	17.35±6.67	8.64	12.66	6.8256	0.0001*
		Post-wash	8.71±11.20				
Total motility	Single IUI	Pre-wash	95.09±7.64	67.71	11.61	58.3113	0.0001*
		Post-wash	27.38±12.94				
	Double IUI	Pre-wash	94.65±5.05	64.95	12.83	50.6119	0.0001*
		Post-wash	29.70±12.51				

[Table/Fig-11]: Comparison of pre and post-wash progressive, pre and post-wash non progressive and pre and post-wash total motility % scores in single IUI and double IUI by Independent t-test.
*p<0.05, SD: Standard deviation

Morphology	Single IUI Mean±SD	Double IUI Mean±SD	t-value	p-value
Normal	9.16±5.07	9.71±6.03	-0.6921	0.4897
Head defect	46.46±15.56	45.98±13.08	0.2386	0.8116
Tail defect	20.68±15.82	16.53±13.30	2.0021	0.0466*
Mid piece defect	22.24±14.82	25.87±13.98	-1.7795	0.0767
Vitality %	64.33±11.43	60.79±13.98	1.9783	0.0500*

[Table/Fig-12]: Comparison of single IUI and double IUI with mean morphology scores by Independent t-test.
*p<0.05 SD: Standard deviation

the pregnancy rates for double IUI in cases of male factor infertility were 19.8%, which is similar to our findings. However, the difference between double and single IUI was more pronounced, with rates of 19.8% and 11.06% (p<0.05) [9]. Similar conclusions were noted by Patil D, where the pregnancy rates were 8% for single IUI and 13% for double IUI [16]. The Cochrane Database of Systematic Reviews reported a pregnancy rate of 14% following single IUI and between 16% and 23% for double IUI, which aligns with our study. However, the quality of evidence was deemed low, and it was concluded that better studies are required for validation [17]. A systematic review and meta-analysis did not support the use of double IUI in clinical practice due to low-quality evidence [18].

There was a significant improvement in pre- and post-wash semen parameters in the semen samples used for IUI, except for total motile sperm count. The present finding is consistent with a study conducted by Ruiter-Ligeti J et al., which noted improvements in all sperm parameters, including increases in sperm concentration, percentage of motile sperm, and forward sperm progression (p=0.0001), but a decrease in total motile sperm count [19]. According to Kastury RD and Taliadouros GS, motile sperm count in unprocessed semen (p=0.0005) and total motile sperm inseminated (p=0.0003) were significantly associated with the occurrence of pregnancy [20]. Higher quality studies with better evidence and larger study populations are required for hypothesis formulation, as the outcomes may differ.

Limitation(s)

The sample size is small, and the duration of the study period is relatively short. In this study, there is no comparison of pregnancy rates achieved with pre- and post-wash semen samples; therefore, the effectiveness of improved post-wash semen parameters in translating into increased conception rates could not be assessed. Although sperm parameters were compared between both groups, the pregnancy rates achieved in each group (parameter-wise) were not determined.

CONCLUSION(S)

Double IUI increases the total concentration of higher quality (post-wash) sperm delivered and extends the window of sperm exposure to the oocyte. This additional procedure may result in increased pregnancy rates. However, in our study, no significant difference was noted in the pregnancy rates achieved.

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