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Research Article

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FemaSeed Directional Intratubal Artificial Insemination for Couples with Male-Factor or Unexplained Infertility Associated with Low Male Sperm Count

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Abstract

Objective: *To report pregnancy outcomes following intratubal insemination without catheterization using the FemaSeed device in a population of low sperm count couples.*

Methods: *A prospective, single-arm, historical control, pivotal trial (Clinicaltrials.gov NCT04968847) of females aged 19-40 years with male-factor or unexplained infertility and total motile sperm count of 1-20 million undergoing intratubal insemination (ITI) with the FemaSeed device, conducted at twelve academic and private medical centers in the USA. Pregnancy was confirmed at 3 weeks (-7 days) by serum β-hCG and follow-up continued at 7 weeks (±7 days) for safety, including pregnancy test by serum β-hCG and ultrasonography.*

Results: *The pregnancy rate for ITI with FemaSeed per subject was 26.3% (95%CI: 13.4–43.1%; n=10/38) and 17.5% per cycle (95%LCB: 7.6%, 95%CI: 5.7–29.4%; n=10/57), which was significantly higher than the performance goal of 7% based on the historical control (one-sided P=0.041). The cumulative pregnancy rates through the first and second ITI cycles were 15.8% (95%CI: 7.4–31.8%) and 30.7% (95%CI: 16.5–52.5%), respectively. Safety reports were consistent with intrauterine insemination (IUI), of note ectopic pregnancy and uterine perforation occurred in 0.5% (95%CI: <0.05–2.5%; n=1/222) and 0.0% of cycles, respectively.*

Conclusion: *Targeted intratubal insemination of washed spermatozoa using the FemaSeed ITI device is a safe artificial insemination technique that demonstrated high effectiveness for couples with male-factor/unexplained infertility associated with low male sperm count. Delivery of washed spermatozoa directly into the utero-tubal ostium and fallopian tube without catheterization likely increases sperm-oocyte interaction, suggestive of improved efficiency over conventional intrauterine insemination particularly for male-factor infertility.*

Keywords: Male-Factor Infertility, IUI, Targeted Fallopian Tube Insemination, Intratubal Insemination, FemaSeed

1. Introduction

Infertility is recognized by the World Health Organization (WHO) as a disease and disability, and affects an estimated 1 out of 6 people globally [1]. Intrauterine insemination (IUI) is one of the older assisted reproduction approaches widely used for patients with mild male-factor infertility, anovulation, endometriosis, and unexplained infertility, whereas *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) are typically used for more severe male-factor infertility [2]. Although IVF and ICSI are highly effective, these treatments are invasive, costly and not easily accessible. For minimally-invasive IUI, success rates are reported for mostly retrospective single center studies and are highly variable, with success affected by infertility diagnosis, semen parameters, sperm preparation, age of female partner, and ovarian stimulation regimens. For approximately 50% of couples, male-factor is a primary or contributing cause of infertility and is the sole cause in up to 30% of cases [3]. The success of IUI is less effective for couples with male-factor infertility described as total motile sperm count (TMSC) of less than 20 million [4,5]. IUI treatments are more cost-effective than IVF for TMSC of greater than 10 million [6].

Intratubal insemination (ITI) is an extension of IUI by depositing a higher concentration of washed spermatozoa directly into the fallopian tube, the site of fertilization. Earlier attempts of ITI utilized transcervical or laparoscopic catheterization of the fallopian tube. Due to technical challenges, including risk of damage to the tubal luminal wall, and varying pregnancy rates, with no effectiveness improvement over IUI reported in a prospective study, it has been largely abandoned by clinicians [7-9]. This study details pregnancy outcomes achieved with transcervical targeted ITI without catheterization using the FemaSeed device in women with at least one patent tube, male-factor and/or unexplained infertility and a low TMSC of 1-20 million.

2. Materials and Methods

2.1 Study Design and Subjects

This prospective, open-label, single-arm trial (clinicaltrials.gov NCT04968847) was conducted under a US Food and Drug Administration (FDA) approved investigational device exemption (G200334) to evaluate the safety and effectiveness of the FemaSeed ITI device, designed to concentrate washed spermatozoa at the utero-tubal ostium and into the fallopian tube. The trial was performed at 12 US tertiary medical centers beginning July 2021. Study enrollment concluded early in November 2023 after receiving FDA 510k clearance in September 2023 [10]. Institutional review board (IRB) approval was obtained for all sites. All subjects provided written informed consent.

2.2 Device and Procedure

The FemaSeed ITI is a sterile single-use transcervical device that delivers washed spermatozoa directly into the utero-tubal ostium and the selected fallopian tube (Fig. 1A) [11]. The guide catheter transits the cervix into the uterine cavity to the fundus (Fig. 1B). The flexible balloon transfer catheter is deployed and exits laterally out of the guide catheter and is advanced a pre-set distance to the selected uterine cavity cornu, whereupon the balloon is inflated with air (Fig. 1C) to prevent retrograde egress and promote forward delivery of the subsequently delivered specimen (Fig. 1D). One or both fallopian tubes can be treated in the same session. Following uni- or bilateral insemination, the transfer catheter is retracted and the device withdrawn.

2.3 Inclusion/Exclusion Criteria

Primary inclusion criteria were: i) female, 19-40 years of age, infertile; ii) met qualifications for intrauterine insemination (IUI) [12]; iii) patent fallopian tube(s) without evidence of hydrosalpinx; and iv) agreeable to methotrexate medical treatment for an ectopic pregnancy diagnosis. Primary exclusion criteria were: i) >3 prior IUI cycles or any prior IVF; ii) difficult cervical visualization or instrumentation of the uterus; iii) current or recent infection of cervix, endometrium, or fallopian tubes; iv) history of ectopic pregnancy or tubal surgery; v) known uterine anomaly or uterine position that would interfere with guide catheter midline fundal placement, access to uterine cornu, or lateral deployment of the transfer catheter; and vi) known allergy or sensitivity to methotrexate.

2.4 Visits

Procedures were conducted according to FemaSeed Instructions for Use [11], with the following parameters documented: i) cycle number; ii) medication use, including ovarian stimulation (e.g., clomiphene citrate, letrozole, menotropins); iii) procedure timing with respect to ovulation and method (e.g., hCG injection, urine luteinizing hormone surge); iv) mature follicle assessment to determine uni- or bilateral insemination; v) sperm analysis; vi) procedure time; vii) inseminate volume; viii) discomfort rating using a visual analog scale; ix) investigator assessment of procedure performance noting any device malfunctions/user error; and x) AEs during the procedure.

Subjects returned 3 weeks (-7 days) after ITI, for i) serum quantitative β-hCG pregnancy test result; ii) subject assessment; and iii) post-procedure AE details. Subjects not pregnant at 3 weeks were given the option to repeat the ITI procedure for a maximum of 6 cycle attempts and rated their satisfaction. Subjects who were pregnant at the 3-week timepoint returned 7 weeks (±7 days) post-procedure for a serum pregnancy test and an ultrasound confirmation of an intrauterine pregnancy. If ectopic pregnancy was diagnosed, the investigator managed the condition.

3.Statistical Analysis

Categorical variables were compared using Fisher exact test. Exact 95% confidence intervals (CI) were calculated for rates/proportions at the subject level; normal approximation 95% lower confidence bounds (LCB) and 95% CI were calculated for the primary effectiveness analysis based on sampling theory [13]. Continuous variables were compared using Student t-test when data were normally distributed and Wilcoxon rank-sum test when not normally distributed. Kaplan-Meier analysis estimated the

cumulative probability of pregnancy at each consecutive cycle, accommodating differing numbers of cycles among subjects, with both 95%LCB and 95%CI calculated using normal approximation, and complementary log-log transformation. P-values <.05 were considered indicative of significant differences, and were one- or two-tailed as indicated. Software was SAS v.9.4 (SAS Institute, Cary, NC).

The original subject population included both female and male infertility factors. However, the FDA approved a protocol change mid-trial to include only couples with male-factor/unexplained infertility and total motile sperm count of 1-20 million. A revised power calculation, based on the null hypothesis that the true PR is ≤7%, tested using a one-sided exact binomial test with α=0.025 and 90% power, required a sample size of up to 214 subjects receiving a total of 214 cycles. The performance goal of 7% was chosen based on the historical control which reported a 6.7% pregnancy rate for couples with male-factor/unexplained infertility and TMSC greater than 1 million (no upper limit), who received natural or stimulated IUI procedures (n=1115 cycles) [14,15].

3.1 Outcome Measures

The primary endpoint was confirmed pregnancy rate (PR) at 3 weeks post-insemination in couples with male-factor/unexplained infertility and TMSC of 1-20 million, who completed at least one procedure. Effectiveness analyses assessed cumulative PR by number of ITI cycles and TMSC subcategory. Safety outcomes included the incidence of ectopic pregnancies and uterine perforations related to the device or procedure, and the existence of any other AEs that were possibly or definitely related to the device and/or procedure.

4. Results

The subject flow is shown in Figure 2. Of the Intention-to-Treat (ITT) subjects, 127 (95.5%) had ≥1 procedure; a cumulative 216 cycles (97.3%) were successfully completed. Of the 133 subjects who had ≥1 procedure attempted, 48.1% (n=64) had no identifiable female infertility factor and were diagnosed as male-factor and/or unexplained causality (Supplemental Table S1). These 64 subjects had a cumulative 108 ITI cycles attempted; 4/64 subjects had failed ITI attempts; 22/64 subjects had TMSC less than 1 million or greater than 20 million; and 38/64 subjects had one or more cycles completed that met the TMSC criterion of 1-20 million, for a cumulative 57 cycles (see Figure 2). This group was classified as the Cohort of Interest in the primary effectiveness analysis (Table 1). The remaining subjects 51.9% (n=69) had one or more female infertility factors and constituted the Residual Cohort.

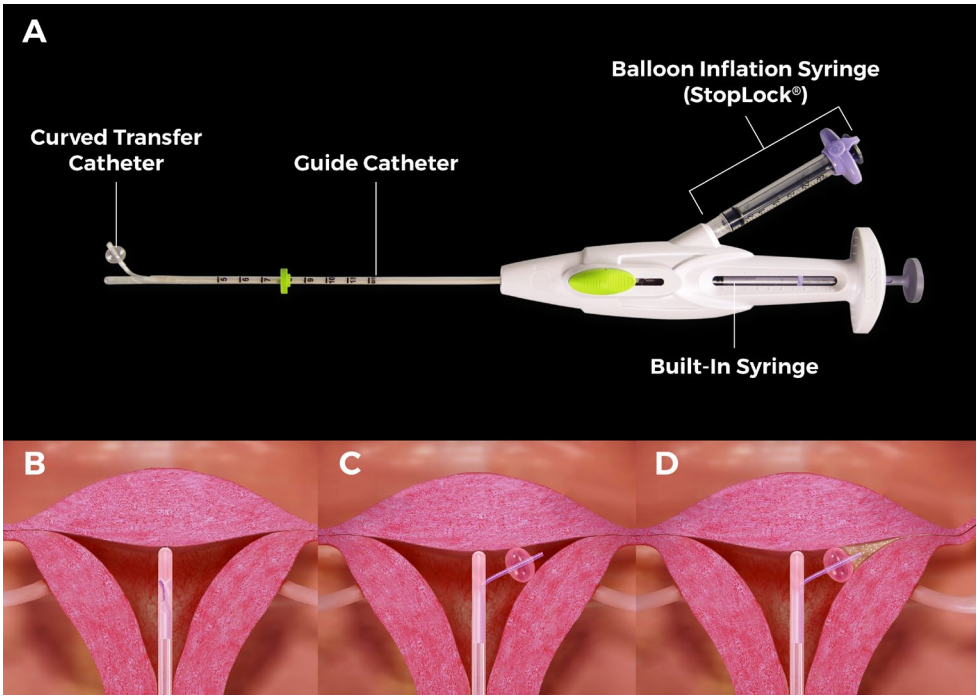

Figure 1: The Intratubal Insemination (ITI) Device.

A) The FemaSeed ITI is a sterile single-use transcervical device that delivers washed spermatozoa directly into the utero-tubal ostium and selected fallopian tube. Following insertion to uterine fundus (B), the flexible balloon transfer catheter exits laterally out of the guide catheter and is advanced a pre-set distance to the selected uterine cavity cornu, whereupon the balloon is inflated with air to seal off the cornu (C). After device placement, the washed spermatozoa sample is delivered directly towards the opening and into the fallopian tube (D). This process can be repeated on the contralateral fallopian tube, if desired.

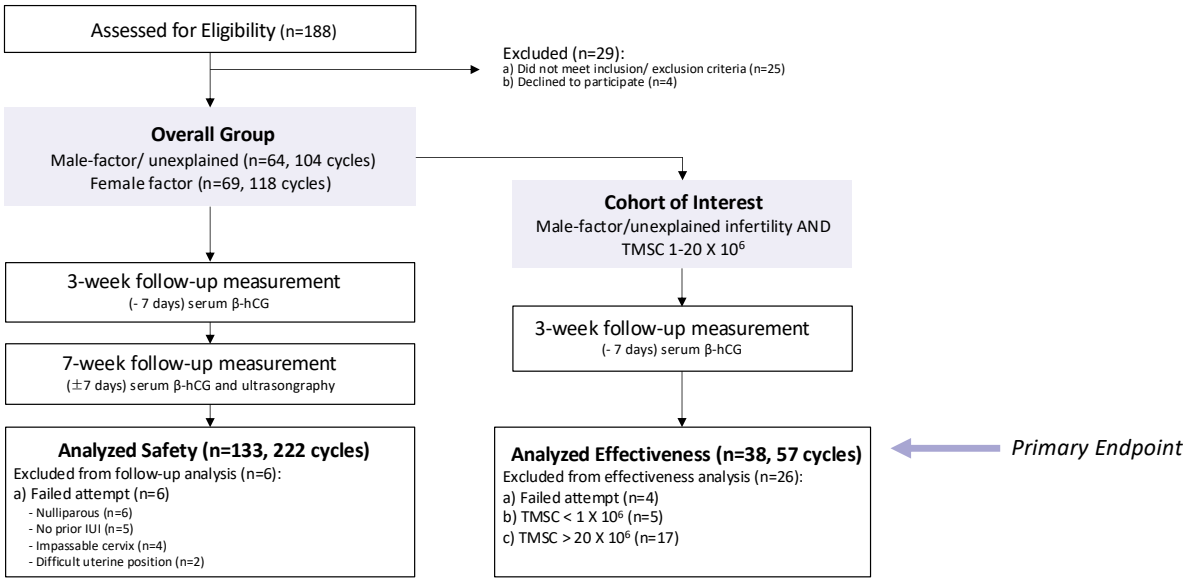

Figure 2: Flowchart of Subject Numbers

Subjects averaged 32 years-old (range 20–40 years), 72.9% were nulliparous with a mean 2.7-year history of infertility, a median BMI of 29.4 (Supplemental Table S1). Approximately 38% of subjects had undergone prior IUI. The majority of subject cycles (90.5%; n=201/222) were preceded by controlled ovarian stimulation and hCG injection (84.7%; n=188) (Supplemental Table S2). Most subject cycles (78.4%) did not receive pre-procedural prophylactic pain or anxiety medication. Semen samples were primarily (88.7% of cycles) partner-sourced. Sperm preparation was equivalently performed by wash-and-centrifugation (46.4%) and density-gradient (47.3%) approaches. The swim-up method was not used. Although a median 2 mature follicles were observed per cycle, there were no multiple pregnancies.

Physicians performed 82.0% (n=182/222) of FemaSeed ITI procedures and advanced practice providers (physician assistants and nurse practitioners) performed 18% (n=40). Almost all (97.3%, n=216) of the ITT procedures were successfully completed with 5/6 failed attempts occurring in nulliparous subjects; 4/6 had an impassable cervix. ITI procedure time (speculum insertion-to-device removal) averaged 7.24 minutes (Supplemental Table S3). When ultrasound guidance was utilized for device placement and/ or sperm delivery, it was predominantly transabdominal (51.4%, n=111). Most (97%) of procedures were completed without user/ device error. One tube was treated in 54.2% of ITI cycles, with a mean delivered sperm volume of 0.38 mL/tube. Very few women required cervical anesthesia (3.6%) or post-procedure medication (2.3%). Procedural details in the male-factor/unexplained infertility subset was similar to those in the ITT population with the main difference being a higher number of sperm delivery to both tubes (58.7% versus 45.8%) and lower use of ultrasound (transabdominal 40.4% versus 51.4%).

4.1 Cohort of Interest

The overall pregnancy rate (PR) in the 38 subjects who met male-factor/unexplained infertility and TMSC criterion (1–20 million) and completed ≥1 FemaSeed ITI cycle was 26.3% (95%CI: 13.4–43.1%; n=10/38 subjects) (Table 1). The per-cycle PR across all 57 FemaSeed ITI cycles performed in this population was 17.5% (95%LCB: 7.6%, 95%CI: 5.7–29.4%; n=10/57) and was statistically significantly greater than the 7% PR performance goal (one-sided P-value=0.041). The PR did not appear to be associated with TSMC level within the trial criterion range of 1-20 million. The estimated cumulative probability of pregnancy after undergoing each ITI cycle per Kaplan-Meier analysis was 15.8% after 1 cycle (95%CI: 7.4– 31.8%) and 30.7% after 2 cycles (95%CI: 16.5–52.5%).

Subject and procedure information for the 10 pregnancies in the Cohort of Interest are provided in Supplemental Table S4. The mean maternal age of subjects who became pregnant was 30.4 years, and the mean paternal age of identifiable sperm sources was 34 years. The majority of subjects were nulliparous (80%), the mean years of infertility was 3.6, 60% conceived on the first ITI cycle attempt, 80% received ovarian stimulation, and 90% had hCG- timed injection. The sperm preparation method was divided equally between wash-and-centrifugation (50%) and density-gradient (50%). Mean (±SD) TSMCs were similar after wash-and-centrifugation ($10.1\pm6.2\times10^6$) and density-gradient ($9.6\pm5.2\times10^6$) preparations. A single tube was targeted for insemination in 40% of women. Of cycles resulting in pregnancy, 30% had been performed by advanced practice providers.

In the Residual Cohort consisting of 69 subjects, 2 had failed attempts with 112 completed cycles performed in 67 subjects diagnosed with ≥1 female factor(s), excluding unexplained; of these, 12 cycles were performed without having TMSC documented. Utility was compromised in women with complicated etiology associated with multiple female factors and/ or low TMSC (≤ 20 million). For women with a single female factor and normal

TMSC (greater than 20 million), the overall PR in this subset was 13.3% (95%CI: 1.7-40.5%; n=2/15 subjects) and a per-cycle PR of 11.1% (95%LCB: 2.0%; n=2/18 cycles) (data not shown).

Of all 133 subjects who had FemaSeed ITI attempted (n=222 cycles) in the safety analysis (Table 2), 15.8% (95%CI: 11.2–21.2%; n=35/222) of cycles reported ≥1 non-serious AE, related to the device or procedure and 0.5% (95%CI: <0.05–2.5%; n=1/222) experienced an SAE, the latter an intraductal ectopic pregnancy identified 34 days after ITI that was successfully resolved by 8 days after methotrexate treatment. The most commonly reported AEs (>1%) related to the device or procedure were pelvic pain (9.0%), vaginal spotting/ bleeding (4.1%), and abdominal cramps (2.3%). The majority (60%) of these events occurred on the day of the treatment and most (97%) within 2 days.

Subject comfort was reflected in self-reported scores at the time of the procedure utilizing a visual analog scale from 0–10, averaging 4.5 discomfort (Table 3). Mean discomfort scores were slightly lower for multiparous (4.1) versus nulliparous (4.6) subjects. The vast majority of subjects (94.3% of cycles) stated they would probably or definitely recommend the FemaSeed ITI procedure to friends and family. Investigator satisfaction was similarly high.

Endpoint / Statistic / Subgroup or Category	**Results**
Pregnancy Rate (per subject)	N=38 Subjects[a]
Overall, % (n/N) [Exact 95% CI]	26.3% (10/38) [13.4–43.1%]
Pregnancy Rate (per cycle)	N=57 Cycles[b]
Overall, % (n/N)	17.5% (10/57)
Cluster sampling estimate (SD) (95% LCB) One-sided P-value [95% CI]	17.5% (6.1) (7.6%) p=0.041 [5.7–29.4%]
TMSC Sub-category, % (n/N) [Exact 95% CI]	
TMSC 1 to 5 million	21.4% (3/14) [4.7–50.8%]
TMSC >5 to 10 million	15.4% (2/13) [1.9–45.5%]
TMSC >10 to 15 million	23.5% (4/17) [6.8–49.9%]
TMSC >15 to 20 million	7.7 % (1/13) [0.2–36.0%]
Cumulative Probability of Pregnancy [c,d]	
FemaSeed Cycle, % pregnancy rate (95% CI) (95% LCB)	
Cycle #1	15.8% [7.4–31.8%] (8.4%)
Cycle #2	30.7% [16.5–52.5%] (18.3%)

Table 1: Effectiveness Assessments in Cohort of Interest (Male-Factor/Unexplained Infertility and TMSC 1–20 million)

CI: Confidence Interval; LCB = Lowest Confidence Boundary; TSMC = Total Motile Sperm Count.

[a] N=38 subjects who had a completed FemaSeed ITI procedure and were classified as male-factor or unexplained for infertility cause with TMSC 1–20 million.

[b] N=57 cumulative FemaSeed ITI cycles completed for 38 subjects, with TMSC level of 1–20 million.

[c] Kaplan-Meier methods were used to estimate the cumulative probability of pregnancy at each cycle.

[d] Results for Cycle #3 through Cycle #5 were omitted due to small numbers of subjects.

Adverse Event Category[a]	Subjects Reporting AE N=133[b]	Cycles with AE N=222[c]
Serious AE, SAE, any, n (%; Exact 95% CI)	1 (0.8%; <0.05–4.1%)	1 (0.5%; <0.05%–2.5%)
SAE, device-related	0	0
Uterine perforation (1º safety outcome)	0	0
SAE, procedure-related	1 (0.8%; <0.05–4.1%)	1 (0.5%; <0.05%–2.5%)
Ectopic pregnancy (1º safety outcome)	1 (0.8%; <0.05–4.1%)	1 (0.5%; <0.05%–2.5%)
Non-serious AE, device- or procedure-related, ≥1% of cycles, n (%; Exact 95% CI)	32 (24.1%; 17.1–32.2%)	35 (15.8%; 11.2–21.2%)
Pelvic pain[d]		20 (9.0%; 5.6–13.6%)
Vaginal spotting or bleeding[e]		9 (4.1%; 1.9–7.6%)
Abdominal cramps[f]		5 (2.3%; 0.7–5.2%)
Any AE, regardless of seriousness or relatedness, n (%; Exact 95% CI)	40 (30.1%; 22.4–38.6%)	44 (19.8%; 14.8–25.7%)

Table 2: Safety Assessment

[a] AEs listed were adjudicated to be possibly or definitely related to the device or procedure.
[b] N=133 total subjects who had an attempted or completed FemaSeed ITI procedure.
[c] N=222 total cycles for subjects who had an attempted or completed FemaSeed ITI procedure.
[d] Includes AEs coded as 'Pelvic pain' and one additional AE coded as 'Procedural pain' which was reported as 'Pelvic pain during procedure'.
[e] Includes AEs coded as 'Vaginal bleeding' and 'Spotting vaginal', as well as one additional AE coded as 'Post procedural bleeding' which was reported as 'Spotting after FemaSeed procedure'.
[f] Includes AEs coded as 'Abdominal cramps' and one additional AE coded as 'Abdominal crampy pains'.

Parameter	N=216 Cycles[a]	N=104 Cycles[a]
Subject-reported discomfort, all[b]		
Mean ± SD	4.5 ± 2.5	5.0 ± 2.4
Median (range)	4.0 (0.0–10.0)	5.0 (0–10)
Subject-reported discomfort, nulliparous[b]	N= 160 Cycles	N=75 Cycles
Mean ± SD	4.6 ± 2.5	5.0 ± 2.4
Median (range)	4.0 (0–10)	5.0 (0–10)
Subject-reported discomfort, multiparous[b]	N=56 Cycles	N=29 Cycles
Mean ± SD	4.1 ± 2.5	4.8 ± 2.6
Median (range)	4.0 (0–10)	6 (0–8)
Subject recommendation to friends and family[c], n (%)		
Yes	124 (58.5)	62 (61.4)
Probably	76 (35.8)	31 (30.7)
No	12 (5.7)	8 (7.9)
Investigator overall satisfaction rating[d]		
Mean ± SD	2.2 ± 0.7	2.3 ± 0.6
Median (range)	2 (1–4)	2 (1–4)
Investigator recommendation to colleague, n (%)		
Yes	91 (42.1)	37 (35.6)
Possibly	106 (49.1)	55 (52.9)
No	19 (8.8)	12 (11.5)

Table 3: Discomfort and Satisfaction Ratings (FemaSeed ITI Completed Cycles Overall and Male-Factor/Unexplained Infertility Subset)

[a] A total of 216 FemaSeed ITI cycles were completed in 127 subjects overall and total of 104 FemaSeed ITI cycles were completed in 60 subjects in the male-factor/ unexplained infertility subset.
[b] Pain assessed using the Wong-Baker FACES® Pain Rating Visual Analog Scale (VAS) score on a scale from 0 to 10.
[c] Subject satisfaction results were queried at 3-week follow-up, before pregnancy status was evaluated. Subject recommendations were available for 212/216 total ITI cycles and 101/104 male-factor/unexplained infertility ITI cycles.
[d] The ratings for Investigator rating of satisfaction and subject tolerability were converted as follows: 1 - Extremely Satisfied, 2 - Very Satisfied, 3 - Neither Satisfied nor Dissatisfied, 4 - Very Dissatisfied, 5 - Extremely Dissatisfied.

Baseline Characteristics	All Subjects	Male-Factor/Unexplained Infertility
Age, years	N=133[a]	N=64[b]
Mean ± SD	32.1 ± 4.4	32.6 ± 3.9
Median (range)	32 (20–40)	33 (24-40)
Ethnicity, n (%)		
Hispanic or Latino	11 (8.3)	6 (9.4)
Not Hispanic or Latino	122 (91.7)	58 (90.6)
Race, n (%)		
American Indian or Alaska Native	1 (0.8)	1 (1.6)
Asian	8 (6.0)	6 (9.4)
Black or African-American	10 (7.5)	4 (6.3)
Native Hawaiian or Other Pacific Islander	1 (0.8)	0 (0)
Other [c]	3 (2.3)	3 (4.7)
White or Caucasian	110 (82.7)	50 (78.1)
Smoker, n (%)		
Never	112 (84.2)	53 (82.8)
Current (within last year)	2 (1.5)	0 (0)
Past	19 (14.3)	11 (17.2)
Weight, pounds, mean ± SD	182.3 ± 48.9	170.6 ± 44.7
Height, inches, mean ± SD	64.7 ± 2.9	65.2 ± 2.9
BMI, lb/in2×703		
Mean ± SD	30.6 ± 7.8	28.2 ± 7.0
Median (range)	29.4 (18.6–58.4)	27.9 (18.6-58.4)
Pregnancy history, n (%)		
Nulliparous	97 (72.9)	46 (71.9)
Multiparous	36 (27.1)	18 (28.1)
Type of infertility, n (%)		
Primary	83 (62.4)	40 (62.5)
Secondary	50 (37.6)	24 (37.5)
Length of infertility, years, mean ± SD	2.7 ± 2.0	2.4 ±2.0
< 1 year, n (%)	9 (6.8)	5 (7.8)
>1-2 years	51 (38.3)	28 (43.8)
>2-3 years	30 (22.6)	16 (25.0)
>3-4 years	19 (14.3)	7 (10.9)
>4-5 years	7 (5.3)	3 (4.7)
>5-10 years	15 (11.3)	3 (4.7)
>10 years	2 (1.5)	2 (3.1)
Cause of infertility, n (%)		
Male factor[d]	26 (19.5)	26 (40.6)
Unexplained	38 (28.6)	38 (59.4)
Male factor plus female factor	6 (4.5)	NA
Female factor, single[e]	15 (11.3)	NA
Female factor, multiple[f]	48 (36.1)	NA
Prior intrauterine insemination, IUI, n (%)	51 (38.3)	24 (37.5)

Supplemental Table S1: Demographic and Baseline Characteristics (ITT Population and Male-Factor/ Unexplained Infertility Subset)

Abbreviations: BMI=body mass index; ITT=intention-to-treat; SD=standard deviation.

[a] N=133 total subjects who had an attempted FemaSeed procedure.

[b] N=64 total subjects who had an attempted FemaSeed procedure and were classified as male-factor/unexplained infertility.

[c] Other includes two subjects that reported multiple races; all remaining categories include a single race.

[d] Includes 1 subject denoted as male factor/unexplained.

[e] Includes 3 categories for single female factor.

[f] Includes 18 categories for more than one female factor

Pre-Procedure Parameter	N=222 Cycles[a]	N=108 Cycles[b]
Controlled ovarian stimulation during cycle, n (%)	201 (90.5)	92 (85.2)
Ovulation predication, n (%)		
hCG injection received	188 (84.7)	91 (84.3)
Ultrasound monitoring	24 (10.8)	17 (15.7)
Ovulation predictor kit	4 (1.8)	0 (0)
Ultrasound monitoring and ovulation predictor kits	6 (2.7)	0 (0)
Pre-medication prior to procedure, n (%)		
Ibuprofen	4 (1.8)	2 (1.9)
Acetaminophen, paracetamol	19 (8.6)	12 (11.1)
Excedrin (acetaminophen+aspirin+caffeine)	1 (0.5)	0 (0)
Diazepam	24 (10.8)	15 (13.9)
Type of sperm utilized, n (%)		
Homologous	197 (88.7)	88 (81.5)
Donor	25 (11.3)	20 (18.5)
Sperm preparation method, n (%)		
Swim-up	0 (0)	0 (0)
Wash & centrifugation	103 (46.4)	47 (43.5)
Density gradient	105 (47.3)	52 (48.1)
Zymot sperm separation device	9 (4.1)	4 (3.7)
Other[c]	5 (2.3)	5 (4.6)
# Mature follicle present		
Mean (SD)	2.3 (1.7)	2.5 (1.6)
Median (range)	2.0 (1–11)	2.0 (1–11)
Mature follicle size (mm)		
Mean (SD)	17.9 (4.5)	18.2 (4.8)
Median (range)	18.0 (2–37)	18.0 (7–37)

Supplemental Table S2: Pre-ITI Assessments by FemaSeed ITI Cycles Performed Attempted (ITT Population and Male-Factor/ Unexplained Infertility Subset)

[a] A total of 222 FemaSeed ITI cycles were attempted in 133 subjects.

[b] A total of 108 FemaSeed ITI cycles were attempted in 64 subjects classified as male-factor/unexplained infertility subset.

[c] Other includes 4 simple wash and 1 thawed frozen, washed and centrifuged. Sperm preparation methods were selected according to investigator preference.

Parameter – All ITI Cycles	N=222 Cycles[a]	N=108 Cycles[a]
FemaSeed ITI Cycle, n (%)		
Completed procedure	216 (97.3)	104 (96.3)
Attempted procedure	6 (2.7)[b]	4 (3.7)[b]
Cervical anesthetic use during procedure, n (%)[c]		
No	214 (96.4)	102 (94.4)
Yes	8 (3.6)	6 (5.6)
Instrument use, n (%)		
Tenaculum	41 (18.5)	31 (28.7)
Dilator	31 (14.0)	21 (19.4)
Parameter – Completed ITI Cycles	N=216 Cycles[d]	N=104 Cycles[d]
Duration of FemaSeed Cycle, min:sec[e]		
Mean (SD)	07:24 (05:29)	07:10 (05:37)
Median (range)	06:00 (02:00–35:00)	05:00 (02:00–35:00)
Ultrasound use, n (%)		
Transabdominal	111 (51.4)	42 (40.4)
Transvaginal	1 (0.5)	0 (0)
# Fallopian tube sperm delivered, n (%)		
One tube only	117 (54.2)	43 (41.3)
Both tubes	99 (45.8)	61 (58.7)
Sperm volume, right tube, mL		
Mean (SD)	0.38 (0.13)	0.38 (0.13)
Median (range)	0.50 (0.10–0.50)	0.50 (0.10–0.50)
Sperm volume, left tube, mL		
Mean (SD)	0.38 (0.13)	0.36 (0.13)
Median (range)	0.50 (0.10–0.50)	0.30 (0.10–0.50)
Medication post-procedure, n (%)[f]		
No	211 (97.7)	103 (99.0)
Yes	5 (2.3)	1 (1.0)

Supplemental Table S3: Procedural Details by FemaSeed ITI Cycles Attempted (ITT Population and Male-Factor/ Unexplained Infertility Subset)

[a] A total of 222 FemaSeed ITI cycles were attempted in 133 subjects overall and 108 FemaSeed ITI cycles were attempted in 64 subjects for male-factor/ unexplained infertility subset.

[b] Six subjects had failed FemaSeed attempt, 5/6 were nulliparous, 5/6 had no prior IUI and 1/6 had 1 prior IUI. Reasons of failure included 4/6 impassable cervix and 2/6 uterine position. Four of these subjects were in the male-factor/ unexplained infertility subset.

[c] Cervical anesthetic included lidocaine and benzocaine topical anesthetic spray.

[d] A total of 216 FemaSeed ITI cycles were completed in 127 subjects overall and 104 FemaSeed ITI cycles were completed in 60 subjects for male-factor/ unexplained infertility subset.

[e] Duration of ITI cycle was determined by procedure stop time (time speculum or device removal from subject, whichever was later) minus procedure start time (time speculum was placed in subject).

[f] Excludes existing medication use.

ID	Age, years (Maternal)	Age, years (Paternal)	Pregnancy History (Nullip/ Multip)	# Years Infertile	Cycle #	Ovarian Stimulation	hCG Injection (type, # hours before cycle)	Total Motile Sperm Count (10^6)	Sperm Prep Method	Pre-Med	Sperm Delivery (one tube/ both tubes)
1	32	34	Nullip	3	2	No	Yes (Letrozole,Menopur)	4.61	Wash & Centrifugation	No	Both
2	29	No data	Nullip	10	5	Yes (Clomid[a])	Yes (Ovidrel[b], 35)	10.37	Density Gradient	Yes (Valium[c])	Both
3	26	53	Nullip	3	1	Yes (Gonal F[d])	Yes (Pregnyl[e], 37)	19.00	Wash & Centrifugation	No	Both
4	34	No data	Nullip	2	2	Yes (Clomid)	Yes (Ovidrel, 35)	13.80	Density Gradient	Yes (Valium)	One
5	39	36	Nullip	10	1	Yes (Letrozolef, Gonal F)	Yes (Ovidrel, 33)	15.00	Density Gradient	No	Both
6	27	27	Multip	1	1	No	Yes (Ovidrel, 35)	2.75	Density Gradient	No	One
7	30	32	Nullip	3	1	Yes (Clomid)	Yes (undisclosed, 36)	13.68	Wash & Centrifugation	No	Both
8	33	33	Nullip	2	2	Yes (Clomid)	Yes (Ovidrel, 34)	6.00	Density Gradient	Yes (Tylenol[g])	Both
9	28	28	Nullip	1	1	Yes (Letrozole,Menopur[h])	Yes (Ovidrel, 35)	4.48	Wash & Centrifugation	No	One
10	26	27	Multip	1	1	Yes (Letrozole,Menopur)	Yes (Ovidrel, 35)	8.75	Wash & Centrifugation	No	One

Supplemental Table S4. Subject and Procedure Details for FemaSeed ITI-Assisted Pregnancies

[a] Clomid = clomiphene citrate; selective estrogen receptor modulator; increases hypothalamic gonadotropin-releasing hormone secretion
[b] Ovidrel = recombinant human chorionic gonadotropin
[c] Valium = diazepam; anxiolytic
[d] Gonal F = recombinant human follitropin-α; follicle-stimulating hormone analogue
[e] Pregnyl = natural urine-derived human chorionic gonadotropin
[f] Letrozole = aromatase inhibitor that boosts follicle-stimulating hormone production
[g] Tylenol = acetaminophen, paracetamol; analgesic
[h] Menopur = natural urine-derived mixture of follicle-stimulating hormone and luteinizing hormone

5. Discussion

Targeted intratubal insemination without catheterization into the fallopian tube by delivery of washed spermatozoa to the utero-tubal ostium using the FemaSeed ITI device resulted in a pregnancy rate (PR) of 26.3% of subjects and 17.5% of cycles with documented male-factor/unexplained infertility and TMSC of 1-20 million. The cumulative probability of pregnancy as calculated by Kaplan-Meier was 15.8% for the first cycle and 30.7% for a second cycle. This demonstrated PR significantly exceeded the historical control PR of 6.7% in a similar patient population (n=1115 cycles, n=332 women) [14,15]. The FemaSeed ITI device was easy to use, associated with high practitioner satisfaction, and with mild discomfort reported by subjects. No uterine perforations were noted, and one serious AE occurred (ectopic pregnancy that was resolved by treatment with methotrexate). Non-serious AEs reported for 15.8% of cycles were primarily low-grade peri-procedural pelvic pain, vaginal bleeding, and abdominal cramping events that resolved quickly without sequelae. Overall, the ITI procedure demonstrated a highly effective PR and an acceptable safety profile in overcoming subfertility associated with male-factor/unexplained couples.

This minimally-invasive directed sperm delivery method into the fallopian tube using FemaSeed differs from earlier, more-invasive, technically challenging and less-successful approaches at intratubal insemination (ITI) that used transcervical or retrograde laparoscopic catheterization of the fallopian tubes [7-9]. Contact between the catheter tip and the fallopian tube luminal surface may induce tubal contractions that could disturb sperm-oocyte interaction or even flush the oocyte out of the fallopian tube [16].

In many IUI studies, the TMSC significantly impacts pregnancy rate in male-factor/unexplained infertility. Most IUI studies are retrospective, single center and vary widely in their design and performance, making it difficult to co mpare findings. The sole retrospective study that comprised natural and controlled ovarian hyperstimulation and included male-factor/unexplained infertility with a post-wash TMSC threshold was selected as a historical

control for this trial [14,15]. That study reported pregnancy rates of 2.1% and 6.7% for TMSC < 1 million and ≥ 1 million respectively. A retrospective single-center assessment of 1039 couples (mean maternal age 32 years) who underwent 3479 IUI cycles (mixed natural cycles and induced ovulation) experienced a 1st-cycle PR of 1.5% with TMSC <10 million versus 10.5% when TMSC was 10–30 million, suggesting 10 million TMSC as a useful threshold when making decisions about treatment with IUI or IVF [6]. Another retrospective report that included all patients who underwent IUI over 14-year period suggested a lower TMSC threshold for IUI in male-factor infertility of 5 million [17]. A more recent 2021 retrospective analysis of 62,758 IUI cycles (mean maternal age 34.5 years) for all infertility factors over a 16-year period identified a gradual decline in PR as TMSC decreased from a plateau level of 16.7% at TMSC ≥9 million to 3–4% below 1 million [18]. We selected 1 million as the lower TMSC limit applied in our prospective trial. American Urological Association and ASRM guidelines indicate that "*Men with low total motile sperm count (<5 million motile sperm after processing) will have limited chances of contributing to a pregnancy rate after IUI"* [19]. In our study, at the lowest TMSC category (i.e., 1–5 million), the PR was 21.4% of cycles. Half of the pregnancies occurred with TMSC <10 million, and 30% of pregnancies occurred in couples with TMSC below the AUA/ASRM effectiveness threshold of 5 million, although our trial was not powered for this type of post hoc analysis. This suggests that the FemaSeed ITI procedure may overcome TMSC thresholds theorized for standard IUI. Thus, ITI may be a useful approach for achieving pregnancy even in couples with severe male-factor subfertility who might otherwise be recommended for costlier IVF or ICSI approaches.

Utilization of advanced practice providers in reproductive medicine increases access to care and reduces costs [20]. Accordingly, 3 of our 12 trial sites (25%) employed physician assistants (PAs) or nurse practitioners (NPs) in addition to medical doctors, who performed a cumulative 18% of ITI cycles in our safety population and accounted for 30% of pregnancies in the cohort with male-factor/unexplained infertility. Our experience is consistent with studies where IUI performed by NPs has a similar likelihood of resulting in pregnancy as IUI performed by medical fellows [21].

Trial limitations were the absence of a concurrent control cohort that received traditional IUI and modest sample size. Comparison of our data to those in the literature can be complicated because of the high degree of heterogeneity in IUI study designs with respect to infertility diagnosis and classification, semen preparation and analysis methods, varying IUI products, and use of divergent ovulation induction protocols. Additionally, many studies of male-factor/unexplained infertility are single center retrospective analyses. Larger randomized multicenter trials that standardize these variables will clarify more precisely the effectiveness outcomes of ITI versus conventional IUI.

In conclusion, the FemaSeed ITI device that targets sperm placement directly into the fallopian tube by concentrating delivery to the utero-tubal ostium offers a safe and effective treatment option to achieve pregnancy in couples with male-factor/unexplained infertility and low male sperm count. For total motile sperm count values of 1–20 million, the device was associated with pregnancy rates of 26.3% by subject and 17.5% by cycle, and the rate per cycle was significantly greater than the performance goal of 7% based on the historical control.

Abbreviations:

ITI – Intratubal Insemination
IUI – Intrauterine Insemination
IVF – In Vitro Fertilization
ICSI – Intracytoplasmic Sperm Injection
CI – Confidence Interval
LCB – Lower Confidence Bound
β-hCG – Beta Human Chorionic Gonadotropin
TMSC – Total Motile Sperm Count
PR – Pregnancy Rate

Declarations

Data Sharing Statement: The anonymized datasets used in the current trial are available from the corresponding author upon reasonable request.
Funding: This trial was supported by Femasys Inc., (Suwanee, GA), the study device manufacturer.
Disclosures: KLS is the founder of Femasys Inc., the study device manufacturer, is an officer and board member of Femasys, has stock and stock options with Femasys, and holds patents associated with the study device. JHL is the Chief Medical Officer for Femasys and receives a stipend for services performed. LM received compensation for statistical services. Authors report no other potential conflicts of interest, financial or otherwise.

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