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RESEARCH PAPER

Effect of autologous bone marrow stem cells-scaffold transplantation on the ongoing pregnancy rate in intrauterine adhesion women: a randomized, controlled trial

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Intrauterine adhesion is a major cause of female reproductive disorders. Although we and others uncontrolled pilot studies showed that treatment with autologous bone marrow stem cells made a few patients with severe intrauterine adhesion obtain live birth, no large sample randomized controlled studies on this therapeutic strategy in such patients have been reported so far. To verify if the therapy of autologous bone marrow stem cells-scaffold is superior to traditional treatment in moderate to severe intrauterine adhesion patients in increasing their ongoing pregnancy rate, we conducted this randomized controlled clinical trial. Totally 195 participants with moderate to severe intrauterine adhesion were screened and 152 of them were randomly assigned in a 1:1 ratio to either group with autologous bone marrow stem cells-scaffold plus Foley balloon catheter or group with only Foley balloon catheter (control group) from February 2016 to January 2020. The per-protocol analysis included 140 participants: 72 in bone marrow stem cells-scaffold group and 68 in control group. The ongoing pregnancy occurred in 45/72 (62.5%) participants in the bone marrow stem cells-scaffold group which was significantly higher than that in the control group (28/68, 41.2%) (RR=1.52, 95%CI 1.08–2.12, *P*=0.012). The situation was similar in live birth rate (bone marrow stem cells-scaffold group 56.9% (41/72) *vs*. control group 38.2% (26/68), RR=1.49, 95%CI 1.04–2.14, *P*=0.027). Compared with control group, participants in bone marrow stem cells-scaffold group showed more menstrual blood volume in the 3rd and 6th cycles and maximal endometrial thickness in the 6th cycle after hysteroscopic adhesiolysis. The incidence of mild placenta accrete was increased in bone marrow stem cells-scaffold group and no severe adverse effects were observed. In conclusion, transplantation of bone marrow stem cells-scaffold into uterine cavities of the participants with moderate to severe intrauterine adhesion increased their ongoing pregnancy and live birth rates, and this therapy was relatively safe.

intrauterine adhesion | Asherman's syndrome | uterine infertility | autologous bone marrow stem cells transplantation | endometrial regeneration | ongoing pregnancy rate

INTRODUCTION

Intrauterine adhesion (IUA), also known as Asherman's syndrome (AS), is a common cause of female reproductive disorders, including amenorrhea, pelvic pain, infertility, recurrent early pregnancy loss, or abnormal placentation (Schenker and Margalioth, 1982; Yu et al., 2008). Histologically, IUA shows endometrium fibrosis (Zhao et al., 2020). Uterine trauma related to pregnancy termination is the most common contributor of IUA, and the prevalence of IUA after dilation and curettage ranges from 15% to 40% (Hooker et al., 2018; Hooker et al., 2014; Salzani et al., 2007; Sroussi et al., 2022). Hysteroscopic adhesiolysis is currently the conventional treatment of IUA (AAGL Elevating Gynecologic Surgery, 2017). However, the recurrence of IUA and endometrium fibrosis often take place (Hanstede et al., 2015; Lin et al., 2015; Vitale et al., 2022; Wang et al., 2022), so the patients face difficulties in achieving pregnancies and avoiding early pregnancy loss especially in moderate to severe IUA (Wang et al., 2022). Therefore, improvement of ongoing pregnancy rate becomes the goal in moderate to severe IUA treatments. In order to prevent IUA recurrence and decrease endometrial fibrosis to achieve pregnancy, several approaches have been investigated, including placing intrauterine device, Foley balloon catheter or hyaluronic acid gel into the uterine cavities following hysteroscopic adhesiolysis (Feng et al., 2023; Lin et al., 2015; Shi et al., 2019; Trinh et al., 2022; Wang et al., 2022); however the effects of these treatments have not been consistently proven in

increasing ongoing pregnancy rate (Bosteels et al., 2017; Vitale et al., 2022; Wang et al., 2022).

As adult stem cells are increasingly understood for treating intractable diseases, we and others explored autologous bone marrow stem cells (BMSCs)-based treatments in IUA to improve the endometrial function (Nagori et al., 2011; Santamaria et al., 2016; Singh et al., 2014; Zhao et al., 2017). However, these studies just enrolled a limited number of participants without controls, although several participants obtaineded promsing fertility outcomes. To demonstrate the efficacy of autologous BMSCs treatment on the ongoing pregnancy rate of the patients with moderate to severe IUA, we conducted this prospective, randomized, controlled clinical trial.

RESULTS

Trial participants

From February 2016 to January 2020, 195 participants were diagnosed as moderate to severe IUA in Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University and Changzhou Maternal and Child Health Care Hospital. Of them, 152 met the inclusion criteria and were randomly allocated to either BMSCs-scaffold group (BMSCs-scaffold plus Foley balloon catheter) or control group (only Foley balloon catheter), with 76 participants in each group. Two participants in BMSCs-scaffold group and three in control group did not receive the allocated treatments after randomization, and two participants did not receive the second-look hysteroscopy in control group. Three and two participants respectively in control group and BMSCs-scaffold group were lost to follow-up in the third menstrual cycle after completing second-look hysteroscopy (Figure 1). Finally, 140 participants were included in the per-protocol set (PPS) for analysis, including 72 participants in BMSCs-scaffold group and 68 in control group, whose primary outcomes were all obtained. In the full analysis set (FAS), 147 participants were included for analysis, containing 74 participants in BMSCs-scaffold group and 73 in control group. In BMSCs-scaffold group, the participants with American Fertility Society (AFS) score (American Fertility Society, 1988) 5–7, 8–10, 11–12 respectively accounted for 23 (31.1%), 46 (62.2%), 5 (6.7%), and in control group the participants with the AFS score 5–7, 8–10, 11–12 separately accounted for 18 (24.6%), 54 (74.0%), 1 (1.4%). There was no significant difference in baseline characteristics in two groups (Table 1).

Primary and secondary outcomes

The primary outcome, ongoing pregnancy, occurred in 62.5% (45/72) in BMSCs-scaffold group and 41.2% (28/68) in control group, and the difference was statistically significant (relative risk (RR)=1.52, 95% confidence interval (CI) 1.08–2.12, P=0.012) until 24 months after first adhesiolysis (Table 2). Of the 45 ongoing pregnancies in BMSCs-scaffold group, 29 (64.4%) were conceived after *in vitro* fertilization (IVF) and 16 (35.6%) were naturally conceived. Of the 28 ongoing pregnancies in control

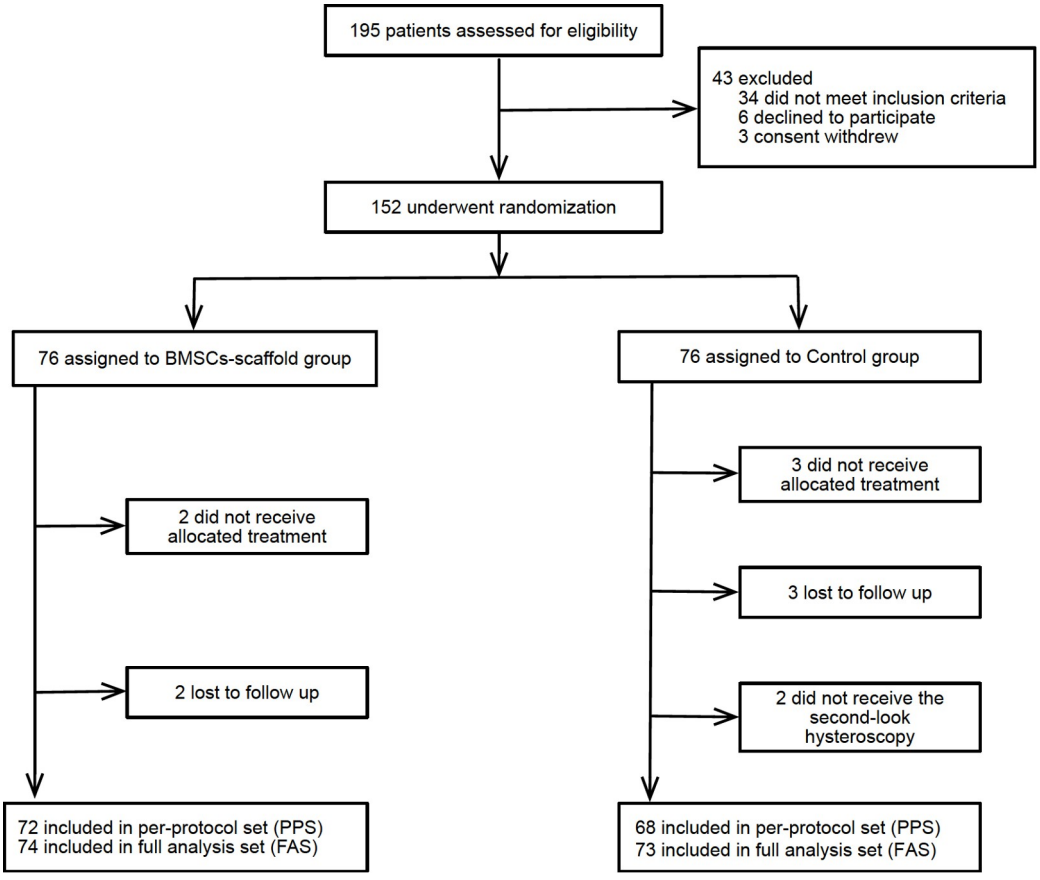

Figure 1. Trial profile.

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Table 1. Characteristics of the participants at baseline[a)]

Characteristic	BMSCs-scaffold group (n=74)	Control group (n=73)	Total (n=147)
Age (years), mean (SD)	30.8 (4.3)	32.0 (4.2)	31.3 (4.2)
BMI (kg m^{-2}), mean (SD)	22.8 (3.3)	22.6 (2.8)	22.7 (3.1)
Gravidity (number), median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)
History of amenorrhea—No. (%)			
No	65 (87.8)	67 (91.8)	132 (90.0)
Yes	9 (12.2)	6 (8.2)	15 (10.0)
Hypomenorrhea—No. (%)			
No	6 (8.1)	8 (11.0)	14 (9.5)
Hypomenorrhea	60 (81.1)	63 (86.3)	123 (83.7)
Spot menstruation	8 (10.8)	2 (2.7)	10 (6.8)
Infertility (years), median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)
Infertility factors—No. (%)			
Only IUA	41 (55.3)	50 (68.5)	91 (61.9)
IUA and tubal factor	13 (17.6)	11 (15.1)	24 (16.3)
IUA and ovulation dysfunction	13 (17.6)	10 (13.7)	23 (15.6)
IUA and male factor	4 (5.4)	2 (2.7)	6 (4.1)
IUA and other factors	3 (4.1)	0 (0.0)	3 (2.1)
Previous separation of IUA—No. (%)			
0	53 (71.6)	53 (72.6)	106 (72.1)
1	15 (20.3)	7 (9.6)	22 (14.9)
2	5 (6.7)	11 (15.1)	16 (10.9)
3	1 (1.4)	2 (2.7)	3 (2.1)
Etiology of IUA—No. (%)			
Pregnancy-related curettage	65 (87.8)	67 (91.8)	132 (89.8)
Tuberculosis	4 (5.4)	3 (4.1)	7 (4.8)
Uterine artery embolization	3 (4.1)	2 (2.7)	5 (3.4)
Nonpregnancy-related curettage	2 (2.7)	1 (1.4)	3 (2.0)
PBAC score pre-operation, median (IQR)	24.0 (19.0–31.0)	25.0 (21.0–29.0)	24.0 (20.0–31.0)
MET pre-operation (mm), median (IQR)	4.8 (4.1–5.5)	4.9 (3.8–6.0)	4.8 (4.0–5.9)
AFS score pre-operation—No. (%)			
5–7	23 (31.1)	18 (24.6)	41 (27.9)
8–10	46 (62.2)	54 (74.0)	100 (68.0)
11–12	5 (6.7)	1 (1.4)	6 (4.1)

a) BMI, body mass index; SD, standard deviation; IQR, interquartile range; PBAC, Pictorial Blood Loss Assessment Chart; MET, maximal endometrial thickness. Summary statistics are presented as n (%) for categorical variables, and as means (SD) or medians (IQR) for continuous variables. IQR denotes interquartile range. There were no significant differences between the two groups in any of the baseline characteristics.

group, 19 (67.9%) were conceived by IVF and 9 (32.1%) were naturally conceived. There was no significant difference in the percentage of IVF between the two groups.

Of 72 participants in BMSCs-scaffold group, 41 (56.9%) had live birth which was significantly higher than that in control group (26/68, 38.2%, RR=1.49, 95%CI 1.04–2.14, P=0.027). In the secondary outcomes, the increase of Pictorial Blood Loss Assessment Chart (PBAC) score (Higham et al., 1990) in the 3rd cycle post-operation was 20.0 (interquartile range (IQR) 13.0–29.0) in BMSCs-scaffold group and 13.0 (IQR 7.0–19.5) in control group (P=0.001), and in the 6th cycle post-operation it was 17.0 (IQR 10.0–26.5) in BMSCs-scaffold group and 9.0 (IQR 5.0–16.0) in control group (P=0.027). The maximal endometrial thickness (MET) in the 6th cycle post-operation was thicker in BMSCs-scaffold group than that in control group (median (IQR) 7.1 (6.2–7.6) *vs.* 6.4 (5.0–7.5), P=0.038). Similarly, more MET increase in the 6th cycle post-operation was found in BMSCs-scaffold group than in control group (median (IQR) 1.9 (1.2–2.7) *vs.* 0.9 (0.5–2.0), P=0.001). The other secondary outcomes, including the rates of IUA recurrence at the second-look, postoperative miscarriage and ectopic pregnancy were listed in Table 2. The efficacy analysis of the FAS was consistent with the result of the PPS (Table 3).

Pregnancy complications and safety outcomes

Both groups did not show statistical difference in preterm birth, fetal growth restriction (FGR), placenta previa and postpartum hemorrhage (Table 4), and no fetus malformations were observed. While BMSCs-scaffold group had more live births,

Table 2. Primary and secondary outcomes of the trial (per-protocol set)[a)]

Outcome	BMSCs-scaffold group (*n*=72)	Control group (*n*=68)	Relative risks (95%CI)	*P* value
Primary outcome				
Ongoing pregnancy—No. (%)	45 (62.5)	28 (41.2)	1.52 (1.08–2.12)	0.012
Secondary outcomes				
Live birth—No. (%)	41 (56.9)	26 (38.2)	1.49 (1.04–2.14)	0.027
PBAC score in the 3rd cycle post-operation, median (IQR)	47.5 (36.0–59.0)	38.5 (29.5–51.0)	–	0.047
PBAC score in the 6th cycle post-operation, median (IQR)	43.5 (32.0–56.0)	36.0 (27.5–46.0)	–	0.037
PBAC score increase in the 3rd cycle post-operation, median (IQR)	20.0 (13.0–29.0)	13.0 (7.0–19.5)	–	0.001
PBAC score increase in the 6th cycle post-operation, median (IQR)	17.0 (10.0–26.5)	9.0 (5.0–16.0)	–	0.027
MET in the 3rd cycle post-operation (mm), median (IQR)	5.5 (4.6–6.4)	5.5 (4.7–6.7)	–	0.847
MET in the 6th cycle post-operation (mm), median (IQR)	7.1 (6.2–7.6)	6.4 (5.0–7.5)	–	0.038
MET increase in the 3rd cycle post-operation (mm), median (IQR)	0.4 (0.0–1.1)	0.3 (0.0–1.0)	–	0.895
MET increase in the 6th cycle post-operation–mm, median (IQR)	1.9 (1.2–2.7)	0.9 (0.5–2.0)	–	0.001
Ectopic pregnancy—No. (%)	3 (4.2)	1 (1.5)	2.83 (0.30–26.58)	0.620
IUA recurrence at the second-look– no. (%)	21 (29.2)	22 (32.4)	0.90 (0.55–1.48)	0.683
AFS score at the second-look, median (IQR)	0 (0–3)	0 (0–3)	–	0.852
AFS score decrease at the second-look, median (IQR)	−7 (−8–−5)	−7 (−9–−6)	–	0.590
Postoperative miscarriage—No. (%)	9 (12.5)	16 (23.5)	0.53 (0.25–1.12)	0.089
Preterm birth (<37GW)—No. (%)	3 (4.2)	3 (4.4)	0.94 (0.18–4.83)	1.000

a) PBAC, Pictorial Blood Loss Assessment Chart; IQR, interquartile range; MET, maximal endometrial thickness; IUA, intrauterine adhesion; GW, gestational weeks. Summary statistics are presented as *n* (%) for categorical variables, and as median (IQR) for continuous variables. IQR denotes interquartile range.

Table 3. Primary and secondary outcomes of the trial (full analysis set)[a)]

Outcome	BMSCs-scaffold group (*n*=74)	Control group (*n*=73)	Relative risks (95%Cl)	*P* value
Primary outcome				
Ongoing pregnancy—No. (%)	45/74 (60.8)	29/73 (39.7)	1.53 (1.09–2.14)	0.011
Secondary outcomes				
Live birth—No. (%)	41/72 (56.9)	27/70 (38.6)	1.48 (1.03–2.11)	0.028
PBAC score in the 3rd cycle post-operation, median (IQR)	46.5 (36.0–59.0)	38.0 (29.0–51.0)	–	0.047
PBAC score in the 6th cycle post-operation, median (IQR)	44.0 (32.0–56.0)	36.00 (28.0–46.5)	–	0.028
PBAC score increase in the 3rd cycle post-operation, median (IQR)	19.5 (12.0–29.0)	12.00 (7.0–19.0)	–	0.001
PBAC score increase in the 6th cycle post-operation, median (IQR)	17.0 (10.0–26.0)	9.00 (5.0–16.0)	–	0.001
MET in the 3rd cycle post-operation (mm), median (IQR)	5.5 (4.6–6.3)	5.5 (4.7–6.7)	–	0.714
MET in the 6th cycle post-operation (mm), median (IQR)	7.0 (6.2–7.6)	6.4 (5.0–7.5)	–	0.051
MET increase in the 3rd cycle post-operation (mm), median (IQR)	0.4 (0.0–1.1)	0.4 (0.0–1.0)	–	0.968
MET increase in the 6th cycle post-operation (mm), median (IQR)	1.9 (1.2–2.7)	0.9 (0.5–2.0)	–	0.001
Ectopic pregnancy—No. (%)	3/72 (4.2)	1/70 (1.4)	2.92 (0.31–27.37)	0.620
IUA recurrence at the second-look—No. (%)	22/74 (29.7)	23/71 (32.4)	0.92 (0.56–1.49)	0.729
AFS score at the second-look, median (IQR)	0 (0–3)	0 (0–3)	–	0.820
AFS score decrease at the second-look, median (IQR)	−7 (−8–−5)	−7 (−8–−5)	–	0.596
Postoperative miscarriage—No. (%)	9/72 (12.5)	17/70 (24.3)	0.51 (0.25–1.08)	0.069
Preterm birth (<37GW)—No. (%)	3/72 (4.2)	3/70 (4.3)	0.97 (0.19–4.98)	1.000

a) IQR, interquartile range; IUA, intrauterine adhesion; GW, gestational weeks. Summary statistics are presented as *n* (%) for categorical variables, and as median (IQR) for continuous variables. IQR denotes interquartile range.

there were also more participants who required manual removal of placenta compared with women in control group (12.5% (9/72) *vs*. 2.9% (2/70), *P*=0.032). There was no difference in neonatal outcomes (Table 4) and safety outcomes (Table 5) in both groups.

Subgroup analysis

Results of prespecified subgroup analyses for the primary outcome are shown in Figure 2. In subgroup analysis, participants with AFS score 8–10 in BMSCs-scaffold group

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Table 4. Maternal and neonatal outcomes of the trial[a)]

Outcome	BMSCs-scaffold group (n=74)	Control group (n=73)	P value
Maternal outcomes			
Placenta accreta—No. (%)	9/72 (12.5)	2/70 (2.9)	0.032
Placenta previa—No. (%)	3/72 (4.2)	0/70 (0.0)	0.245
Postpartum hemorrhage—No. (%)	2/72 (2.8)	1/70 (1.4)	1.000
Cervical incompetence—No. (%)	2/72 (2.8)	2/70 (2.9)	1.000
Neonatal outcomes#	(n=41)	(n=27)	–
Gestational age at delivery			
Median (IQR) (GW)	39.0 (38.0–39.0)	39.0 (38.0–39.0)	0.210
Distribution (<37 GW)—No. (%)	3 (7.3)	3 (11.1)	0.675
Fetal growth restriction—No. (%)	0 (0.0)	0 (0.0)	–
Neonatal asphyxia—No. (%)	0 (0.0)	0 (0.0)	–
NICU admission—No. (%)	0 (0.0)	0 (0.0)	–
Delivery method (cesarean)—No. (%)	21 (51.2)	10 (37.1)	0.251
Newborn sex (male)—No. (%)*	20 (46.5)	16 (57.1)	0.381
Newborn weight (kg), median (IQR)*	3.4 (2.9–3.6)	3.3 (3.1–3.5)	0.343

a) GW, gestational weeks; IQR, interquartile range; NICU, neonatal intensive care unit. # Newborns were born to 27 participants in the control group and 41 participants in the BMSCs group. * There was one twin in control group (n=28) and two twins in BMSCs group (n=43).

Table 5. Safety outcomes of the trial

Safety outcomes	BMSCs-scaffold group (n=74)	Control group (n=73)	P value
Adverse events			
Bonemarrow puncture failure—No. (%)	0 (0.0)	0 (0.0)	–
Postoperative lower abdominal pain—No. (%)	9 (12.2)	7 (9.6)	0.618
Postoperative fever—No. (%)	0 (0.0)	0 (0.0)	–
Abnormal vaginal bleeding—No. (%)	0 (0.0)	0 (0.0)	–
Infection—No. (%)	0 (0.0)	0 (0.0)	–
Anesthesia accident—No. (%)	0 (0.0)	0 (0.0)	–
Allergic reaction—No. (%)	0 (0.0)	0 (0.0)	–
Laboratory testing			
Leukocyte count (abnormal)—No. (%)	2 (2.7)	3 (4.1)	0.681
Neutrophil percentage (abnormal)—No. (%)	5 (6.8)	7 (9.6)	0.531
Neutrophil count (abnormal)—No. (%)	3 (4.1)	5 (6.9)	0.494
C-reactive protein (increase)—No. (%)	0 (0.0)	0 (0.0)	–
Serious adverse events			
Perforation of uterus—No. (%)	0 (0.0)	0 (0.0)	–
Fetus malformations—No. (%)	0 (0.0)	0 (0.0)	–

showed a higher ongoing pregnancy rate (63.0% (29/46)) than that in control group (40.7% (22/54)), and the difference was statistically significant (RR=1.55, 95%CI 1.05–2.29). The similar tendency was found in participants with AFS score 5–7. The ongoing pregnancy rate was 65.2% (15/23) in BMSCs-scaffold group, which was higher than 38.9% (7/18) in control group, although there was no statistical difference between the two groups (RR=1.68, 95%CI 0.87–3.22). The results of RR on primary outcomes in the prespecified subgroups were consistent with the overall results (Figure 2).

Sensitivity analyses

The trial was conducted at two centers, one of which enrolled only 8 women. A post-hoc sensitivity analyses (Table S1 in Supporting Information) excluding the 8 women who recruited at this center yielded the similar results to which including all participants analysis. The primary outcome, ongoing pregnancy, occurred in 62.9% (44/70) in BMSCs-scaffold group and 39.1% (27/69) in control group (RR=1.55, 95%CI 1.11–2.17, P=0.008).

DISCUSSION

The purpose of IUA treatment is to restore normal anatomical uterine morphology and improve endometrium function so as to increase patients' reproductive performance. In this study, we demonstrated that transplantation of autologous BMSCs-scaffold

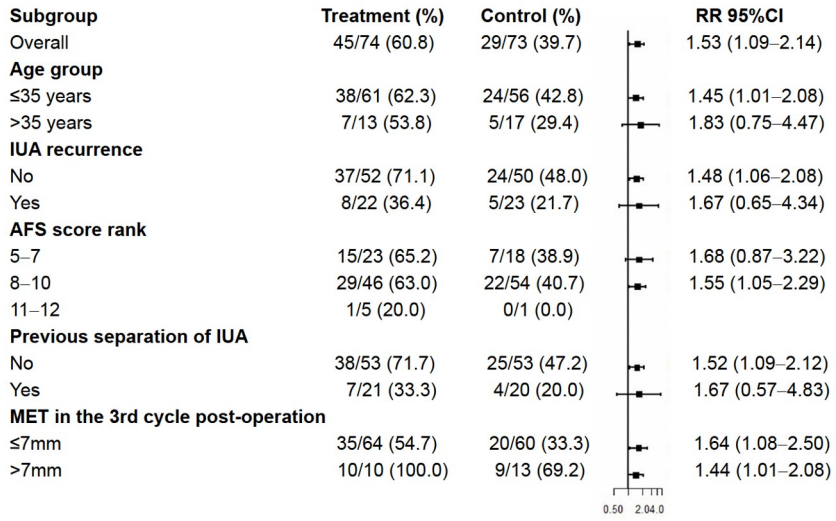

Figure 2. Results of prespecified subgroup analyses for the primary outcome. (The numerator indicates the number of ongoing pregnancies, and denominator, subtotal number of participants. RR represents the relative risk, which is the ratio of ongoing pregnancy rate between the BMSCs-scaffold group and control group. The squares represent the risk ratio of BMSCs-scaffold group versus control group. The error bars show 95% confidence intervals for RR of the primary outcome.)

reconstructed endometrial function and improved the ongoing pregnancy rate of the patients with moderate to severe IUA. To our knowledge, this is the first randomized clinical trial with the largest samples of IUA participants to assess the reproductive outcomes of autologous BMSCs-based therapy comprehensively up to now.

Traditionally, Foley balloon catheter is used to prevent IUA reformation after adhesiolysis surgery (AAGL Elevating Gynecologic Surgery, 2017), so we used Foley balloon catheter alone as a control intervention. Previous studies reported that the placement of Foley balloon catheter in the uterine cavity lasted for 3–7 days (Myers and Hurst, 2012; Lin et al., 2015), or even longer (Chen et al., 2017; Wang et al., 2022). In our study, the Foley balloon catheter in control group was placed in the uterine cavity for 72 hours. In BMSCs-scaffold group, we used Foley balloon catheter to deliver BMSCs-scaffold into the uterine cavity, and we just left it in the uterine cavity for 24 hours to minimize the potential effects of Foley balloon catheter on observing the effects of BMSCs-scaffold. There is little risk of BMSCs-scaffold dropping out after balloon removal, and whether the detachment of BMSCs-scaffold has a negative impact on BMSCs therapeutic effects needs further research. In this trial, none of BMSCs-scaffolds fell off after removing Foley balloon catheter in BMSCs-scaffold group. Therefore, the higher ongoing pregnancy and live birth rates in BMSCs-scaffold group indicate that the better fertility effects are mainly related to BMSCs-scaffold treatment.

In further analysis, it showed that more ongoing pregnancies and live births occurred in participants with AFS score 8–10 in BMSCs-scaffold group than in control group, which not only suggested that the BMSCs-scaffold treatment to IUA patients was superior to traditional treatment but also indicated that BMSCs-scaffold treatment was more appropriate for patients with AFS score 8–10 (Figure 2). The effects of BMSCs-scaffold were also reflected in improvements of menstrual blood volume and endometrial thickness. We also found that the improvement of clinical manifestations after BMSCs-scaffold treatment maintained to six menstrual cycles post-therapy (Table 2), which was longer than that in other reports (Santamaria et al., 2016; Singh et al., 2020), and that may be attributed to the novel method of cell therapy we used. Different from other reports, we adopted the scaffold to load BMSCs and then transplanted it into uterine cavity in this study, which could keep the BMSCs survive and gather at the site of injuries to exert their therapeutic effects (Ding et al., 2014; Su et al., 2016; Tang et al., 2022; Xu et al., 2017; Wang et al., 2021). Moreover, the use of Foley balloon catheters can facilitate the delivery of BMSCs-scaffold into the uterine cavity easily and make it close contact with the endometrium.

Placenta accrete is a common complication if IUA patients successfully become pregnant. In this study, the incidence of placenta accrete in both groups is higher than that in pregnant women without IUA (Mogos et al., 2016). More women in BMSCs-scaffold group (12.5%, 9/72) underwent manual removal of placenta than in control group (2.9%, 2/70) (Table 4), although it was a mild type in placental acceta spectrum. Since placenta accrete is mainly due to decidual dysplasia, high placenta accrete suggests that the endometrial function after one therapy may not completely recover. It merits further investigation that whether two or more BMSCs therapies could reduce placenta accrete. Although there are few other obstetric complications, further study is needed to evaluate obstetric complications comprehensively.

Our study had several strengths. First, this is the first prospective randomized controlled clinical trial including 140 IUA participants with a long follow-up period (2 years) of reproductive outcomes and a very low rate of missing follow-up. Second, all surgical procedures were completed by four experienced gynecologists who strictly adhered to the protocols with a high degree of homogeneity. Third, we used the ongoing pregnancy rate as the primary outcome, which was an objective and reliable indicator. Our study also had some limitations. First, the trial included only two centers, and the second center enrolled only 8 women. However, the result of post-hoc sensitivity analyses after excluding these 8 women did not affect overall outcomes. Second, this study included only 6 participants with AFS score 11–12 in two groups; hence the therapeutic effects on such participants are unknown yet. Third, 90.0% IUA

participants in this study were due to the pregnancy-related curettage, so the effects of BMSCs therapy on endometrial function in IUA caused by other factors need further investigation.

In summary, the transplantation of BMSCs-scaffold into uterine cavities is safe and effective in increasing ongoing pregnancy and live birth rates for moderate to severe IUA patients, especially with AFS score 8–10. Further research is needed to evaluate whether there is a time-dependent phenomenon in BMSCs transplantation and assess the benefits of more times of BMSCs-scaffold treatments.

MATERIALS AND METHODS

Study design

The project was an open-label, randomized clinical trial to compare the efficacy of transplantation of autologous BMSCs-scaffold plus Foley balloon catheter treatment to only Foley balloon catheter treatment following hysteroscopic adhesiolysis on the ongoing pregnancy rate in moderate to severe IUA participants at two hospitals in China. The trial protocol was approved by the institutional review boards and registered at ClinicalTrial.gov (registration number: NCT 02680366). In each of the study units, the data monitoring was conducted by two dedicated physicians who had received good clinical practice training. Written informed consents were provided by all participants. All the authors vouched for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

Trial participants

The participants in this trial were recruited from February 2016 to January 2020 at the departments of obstetrics and gynecology in Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China and Changzhou Maternal and Child Health Care Hospital, Changzhou, China. The inclusion criteria were following: women aged 20–40 years, having reproductive intention, moderate to severe IUA based on AFS scoring (AFS score 5–12) (American Fertility Society, 1988), having a normal ovarian function, and body mass index (BMI) <30 kg m^{-2}. The exclusion criteria included thin endometrium without adhesion, congenital uterine malformations, contraindications to bone marrow collection or pregnancy, contraindications to hormonal treatment, the history of more than 3 times of hysteroscopic adhesiolysis before, chromosome karyotype abnormalities, adenomyosis or myoma with an enlarged uterus (more than 50 days gestational size) and medical history of the malignant tumor. After consent conversation, the subject willing to participate in this program would take the screening tests, and the results would be recorded.

Randomization

All eligible participants were informed about the trial by the physicians. After they provided written informed consents, the participants were randomly assigned in a 1:1 ratio to either BMSCs-scaffold group or control group. Randomization lists were generated by an independent statistician using a randomization program with varying block sizes of four or six. Randomization was performed by the investigators using random envelopes to conceal the study group allocation. All participants group assignments were known one day before surgeries by opening the random envelopes. Owing to the apparent difference in treatment methods between two groups, the trial was unlikely to be blinded for the participants and investigators.

Preparation of the BMSCs-scaffold

For BMSCs-scaffold group, approximately 80 mL of bone marrow was collected in heparinized tubes from 4–5 different puncture points under local anesthesia on the day of hysteroscopic adhesiolysis. After dilution with saline at 1:1 ratio, BMSCs were separated with human mononuclear separating solution (Tianjin Haoyang, Clinical Grade, Tianjin, China). The BMSCs were diluted to a final volume of 1 mL. The viability of the BMSCs was determined by counting the propidium iodide (PI) positive cells via PI-staining. The cell viability is more than 95% (Zhao et al., 2017). The commercial clinically used collagen scaffolds were provided by Zhenghai Biotechnology Company (Yantai, China). As in our previous work, BMSCs were dropped uniformly onto a 4 cm×6 cm scaffold (1×10^6 BMSCs cm^{-2}) and then they were incubated in a 5% CO_2 incubator at 37°C for 45 min before transplantation.

Operation intervention protocols

Four experienced gynecologists (three in Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University and one in Changzhou Maternal and Child Health Care Hospital) were trained together before the trial started. The same surgical procedure was used to separate IUA of the participants in BMSCs-scaffold group and control group on the 10th day taking estradiol (Progynova, Bayer, Germany) 6 mg daily from the third day of menstruation. During the operation, non-energetic microscissors were applied until the uterine cavities returned to normal anatomy. Immediately after adhesiolysis procedure, the BMSCs-scaffold was spread onto an 18F Foley balloon catheter and then inserted into the uterine cavity with 3 mL of sterile saline infilling the balloon to make the scaffold contact the endometrium well confirmed by ultrasonography (Zhao et al., 2017) in BMSCs-scaffold group. 24 hours later, the BMSCs-scaffold was left in uterine cavity after withdrawing the catheter. In control group, only an 18F Foley balloon catheter was used as a barrier between uterine anterior and posterior walls (correct position shown by ultrasonography), and it was removed after 72 hours.

Standard care and follow-up

After the completion of hysteroscopic operations, both groups were treated with oral antibiotics (Levofloxacin, Daiichi Sankyo Company, Japan) for 3 days, vaginal sildenafil citrate (Viagra, Pfizer, USA) 100 mg d^{-1} for 5 days (Sher and Fisch, 2002) and continued taking estradiol 6 mg d^{-1} for the other 40 d with adding progesterone (Duphaston, Abbott, Netherlands) 10 mg d^{-1} for the last 10 days. Such sequential estradiol and progesterone treatment was repeated on the third day of the next menstruation. Menstrual volumes scores were recorded based on PBAC (Higham et al., 1990), and MET was monitored by vaginal ultrasonography in the 3rd and 6th menstrual cycles at the late

proliferation phase (the maximum follicle diameter is 17–20 mm showed in ultrasound) after initial hysteroscopic adhesiolysis. The second-look hysteroscopy was provided to evaluate re-adhesion in natural cycle at the late proliferation phase after 2 hormonal replacement cycles. If there was no re-adhesion observed in the second-look, the participants were given pregnant advice according to their fallopian tubal condition or embryo availability to choose spontaneous conception or *in-vitro* fertilization-embryo transfer. If there was adhesion reformation, adhesiolysis was performed with nothing inserted into the uterine cavity, followed by a third-look hysteroscopy after three menstrual cycles at the late proliferation phase, and then the participants were advised to prepare for pregnancy. After completion of above treatments, the participants were followed up every three to six months by telephone or clinic visits up to 24 months after the initial adhesiolysis, recording menstrual volumes, MET, additional hysteroscopy, pregnancy outcomes, delivery at gestational weeks (GW), spontaneous abortion, neonatal birth weight, fetus malformations, pregnancy and neonatal complications (Figure S1 in Supporting Information).

Outcomes

The primary outcome was ongoing pregnancy rate (the fetus' heartbeat shown at least until 12 GW (Dreyer et al., 2017)). The secondary outcomes included (i) menstrual volumes and menstrual improvement: PBAC scores in the 3rd and 6th menstrual cycles after the initial adhesiolysis and PBAC scores increase compared with pre-operation; (ii) MET and MET increase: MET in the 3rd and 6th menstrual cycles after the initial adhesiolysis in natural period at late proliferation phase monitoring by ultrasonography and MET increase compared with pre-operation; (iii) IUA recurrence rate at the second-look hysteroscopy; (iv) AFS score at the second-look hysteroscopy and AFS score decrease compared with pre-operation; (v) miscarriage rate, preterm birth rate, ectopic pregnancy rate, and live birth rate.

The pregnancy complications were recorded, mainly including placenta previa, placenta accreta, postpartum hemorrhage, cervical incompetence and the rate of FGR. We also recorded neonatal birth weight, sex, delivery method, neonatal asphyxia and neonatal intensive care unit (NICU) admission. The safety outcomes included the complications associated with hysteroscopic adhesiolysis and bone marrow collection.

Statistical analysis

According to the pregnancy rate in moderate to severe IUA cases after hysteroscopic adhesiolysis in previous studies and the high likelihood of early pregnancy loss in IUA patients, we predicted that the ongoing pregnancy rate was about 10% (Amer et al., 2010) in control group and 30% (Santamaria et al., 2016; Singh et al., 2020) in BMSCs-scaffold group. To accept a Type 1 error of 0.05 and a Type 2 error of 0.2, the number of participants required to show significant difference in each group must be 59. Assuming a 20% dropout rate, a total of 72 participants were recruited in each group.

The statistical analysis plan was designed by independent statisticians prior to database locking and approved by the institutional review boards. Efficacy analysis was performed on the following sets: (i) PPS (per-protocol set); (ii) FAS (full analysis set). PPS included all participants who met eligibility criteria, complied with the protocol, received the allocated treatment and completed the assessment period. Missing data in all variables were not imputed in the PPS. The FAS was performed on the intention-to-treat (ITT) principle, which included all participants who are randomized according to the groups they were originally assigned, regardless of what treatment (if any) they received. Missing primary outcome data was imputed with worst-case in FAS.

The primary efficacy hypothesis was that BMSCs-scaffold group would be superior (statistical superiority) to control group in improving the ongoing pregnancy rate. To satisfy the superior hypothesis, the RR and 95%CI were calculated for the primary and other binary outcome measures, the lower boundary of CI of RR had to exceed 1.0.

Prespecified subgroup analyses of the primary outcome were conducted; subgroups were defined according to age-group, IUA recurrence, AFS score rank, previous separation of IUA, MET in the 3rd cycle post-operation. For post-hoc sensitivity analyses, the primary analysis plan was repeated, then we assessed the center effect on the effectiveness and safety outcomes of trial intervention by excluding the second center participants from FAS.

Categorical data was described as absolute numbers and percentages. Normal distributed continuous variables were reported as means with standard deviations, and nonnormally distributed continuous variables were summarized as medians with an interquartile range. Category outcomes were analyzed with the Chi-square test or Fisher test, and continuous outcomes were analyzed with *t*-test or the Mann-Whitney *U*-test as appropriate. Two-sided *P* values of less than 0.05 were considered as statistical significance. R software (version 3.54) was used for statistical analyses.

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Compliance and ethics

The author(s) declare that they have no conflict of interest. For studies of human participants, we conformed with the Helsinki Declaration of 1975 (as revised in 2008) concerning Human Rights, and that we followed out policy concerning Informed Consent as shown on Springer.com.

Supporting information

The supporting information is available online at https://doi.org/10.1007/s11427-023-2403-7. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

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