Uterine size and volume are associated with higher live birth rate in patients undergoing assisted reproduction technology
A prospective cohort study

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Abstract
To investigate how uterine size and volume are associated with live birth rate in patients undergoing assisted reproduction technology.

This prospective cohort study was conducted at the Reproductive Medicine Centre from January 2010 to May 2017. Multivariate binary logistic regression was used to evaluate the relations between uterine size, total volume, and live birth outcomes, after they were adjusted for the main influencing factors.

A total of 7320 women of clinical pregnancy were enrolled. Compared with uterine lengths of 50 to 59 mm (referent), women with uterine lengths ≥60 mm had a lower live birth rate (RR = 1.541). Compared with uterine widths of ≥50 mm (referent), women with uterine widths <30 mm had a lower live birth rate (RR = 1.454). Compared with uterine anteroposterior diameters of <30 mm (referent), women with uterine anteroposterior diameters ≥50 mm had a lower live birth rate (RR = 1.636). Compared with uterine volumes of 30 to 49 mL (referent), women with volumes <30 mL and ≥70 mL had lower live birth rates (RR = 1.368 and 1.742, respectively).

Our findings indicate that uterine sizes and volumes that were too large or too small reduced the live birth rate.

Abbreviations: ART = assisted reproduction technology, E<sub>2</sub> = estradiol, FSH = follicle-stimulating hormone, HCG = human chorionic gonadotropin, HMG = human menopausal gonadotropin, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, RR = relative risk.

Keywords: ART, female infertility, live birth rate, uterine size, uterine volume

1. Introduction
For patients and gynaecologists, the most important objective is having the birth of at least one live-born healthy baby. A recent study reported that 803,792 babies were born worldwide following ART from 2008 through 2010, and a total of >4,461,309 ART cycles were initiated. From these results, we found that not every cycle of ART treatment was successful, and the birth rate was not high. Live birth outcomes may be influenced by many factors, such as maternal age, response to ovarian stimulation, and embryo parameters. However, there have been few studies addressing the association between the uterus and live birth outcomes following ART.

The uterus has the most basic and important function, which is to nourish and protect the developing foetus during pregnancy until birth. Accordingly, uterine size, volume, and endometria all influence the implantation of the embryo and the growth and development of the foetus. At present, existing studies examine the relationships between congenital uterine anomalies and ART outcome, <sup>[11–14]</sup> uterine size and volume, <sup>[11–14]</sup> and uterine immune profiling, and live birth rate. <sup>[13]</sup> Our previous studies have shown that the size and volume of the uterus can affect clinical pregnancy rates in ART patients. <sup>[14]</sup> The optimal uterine size and volume led to the highest clinical pregnancy rates. It is well known that pregnant women still have a long way to go from clinical pregnancy to live birth. During this period, the fetus lives in the uterus. Therefore, it is conceivable that the size and volume of the uterus are important for live-born babies. Nevertheless, studies of the relationships between uterine size (including length, width, anteroposterior diameter), and total volume and live birth outcome following ART in infertile women are rare.

We attempted to find the size and volume range of the uterus that is detrimental to the growth and development of the foetus in...
the uterus. We hypothesized that uterine size and volume are associated with the live birth rate in infertile Chinese Han women.

2. Materials and methods

This prospective cohort study was conducted among women undergoing ART treatments in the Reproductive Medicine Centre, all of whom provided informed consent. This study was performed in accordance with all relevant guidelines and regulations. The Reproductive Medicine Centre of Xiangya Hospital is an important integrated ART treatment centre in south China that drew patients from all over China.

2.1. Study population

All women presenting to the Reproductive Medicine Centre, Xiangya Hospital of Central South University, Hunan, China, who were planned for ART treatments and who signed the informed consent were enrolled in our study from January 2010 to May 2016. Exclusion criteria included women with congenital uterine malformation, uterine septum, uterus duplex, uterine cancer, rudimentary horn of uterus, hysterosomyoma, adenomyosis, intrauterine adhesions or all of the uterus after surgical operation. All women with clinical pregnancy (ultrasound confirmed an intrauterine gestational sac) by ART treatments were included in our final analysis.

2.2. Data collection

All data of the subjects were recorded by the electronic medical records (Haitai, Nanjing, China) of Xiangya Hospital Central South University. The subjects were followed up for 12 months with the last visit in May 2017. All subjects were interviewed face to face, collecting their data, including age, social-demographics, history of assisted reproductive technology, and treatment outcomes of assisted reproductive technology. Height, weight, and BMI were measured. Pre-cycle uterine size was measured by transvaginal ultrasonography. Information about artificial insemination and live birth outcomes was collected. The data recorders included attending gynaecologists, fellows and nurses.

2.3. Uterine size measurements

The uterine size of all patients was measured by transvaginal ultrasonic image examination. All ultrasonic scans were performed with 5.0 to 8.0MHz scanners (DC – 6 Expert, Mindray) by gynaecologists specialized in gynaecological ultrasonography. In our study, the three most senior gynaecologists who were specialists in gynaecological ultrasonography took the uterine measurements. By 2011, they had at least 16 years of work experience; so, the uterine measurement results were reliable. The following parameters were analyzed: uterine longitudinal diameter (length), transverse diameter (width), and anteroposterior diameter. The longitudinal diameter was measured from the cervical internal os to the fundus in the sagittal plane; the transverse diameter was measured by the maximum diameter from the right to the left side of the uterine corpus in the transverse plane; the anteroposterior diameter was measured from the anterior serosa to the posterior serosa at the point at which the uterus appeared at its thickest and perpendicular to the endometrial line in the sagittal plane. Uterine volume was calculated according to the formula for ellipsoid bodies: $V = \text{longitudinal diameter} \times \text{anteroposterior diameter} \times \text{transverse diameter} \times 0.5233$.\(^{118}\)

2.4. ART treatment

The protocols of ovarian stimulation mainly included a long, short, antagonist, and mini-stimulation protocol.

Long protocol: on day 21 of the menstrual cycle, patients were injected with gonadotropin releasing hormone agonist (triptorelin), 0.05 to 0.1mg/day, and continued treatment until the day of human chorionic gonadotropin (HCG) release. After reaching the standards of downregulation (FSH $< 5IU/L$, E2 $< 50pg/mL$, LH $< 5IU/L$, follicular diameter $<8mm$, and endometrium $<5mm$), gonadotropin (Gn) stimulation was started, human menopausal gonadotropin (HMG, Menogen; Ferring) or recombinant FSH (rFSH, Gonal-\(f\); Merck Serono, Darmstadt, Germany) 150 to 300 IU/day. The dose of gonadotropin was adjusted according to follicular growth, endometrial thickness, and serum sex hormone levels. After 5 days, the growth and development of the follicle and endometrium were monitored daily or on alternate days with transvaginal ultrasonography. When B ultrasonography showed the mean diameter of 1 to 2 follicles $\geq 20mm$, or 2 and over follicular diameter $\geq 18mm$, then patients were injected with HCG (Livzon, Guangdong, China) at 10,000 IU.

Short protocol: on day 1 to 3 of the menstrual cycle, patients were injected with gonadotropin releasing hormone agonist (triptorelin), 0.05 to 0.1mg/day, and continued treatment until the day of HCG. On day 3, Gn stimulation was started with HMG or rFSH 225 to 300 IU/day. Follow-up treatment was the same as the long protocol.

Antagonist protocol: on day 2 of the menstrual cycle, patients were injected with rFSH daily. After reaching at least one standard (1 and over follicular diameter $\geq 14mm$, E2 $\geq 600pg/mL$, and LH $\geq 10IU/L$), cetrorelix (Cetrotide; Merck Serono) treatment was started, 0.25mg/day. Then, the antagonist and rFSH were used every day until the day of HCG release.

Ovum pickup was conducted 36 to 38h after HCG injection under transvaginal ultrasonography guidance.

The oocytes were evaluated in metaphase II. The selection of fertilization methods relied on the patients’ partner’s condition and mainly included IVF and ICSI. The quality of the embryo was assessed according to multinucleation, the degree of fragmentation and the number of blastomeres.\(^{119}\) The number of embryo transfers was not more than 3. Types of embryo transfer included fresh and frozen–thawed embryos.

2.5. Outcome measures

Live birth means the birth of at least one live-born baby per initiated cycle or embryo transfer procedure, and all the other adverse pregnancy outcomes belong to non-live birth.

2.6. Statistical analysis

All data were managed and analyzed using the statistical package for social sciences (SPSS) software version 17.0 (SPSS Inc 2008, Chicago, IL) and Excel (Microsoft Corp., Redmond, WA). Measurement data were described by mean $\pm$ standard deviation (SD), and enumeration data were described by number (percentage). Multivariate binary logistic regression was used
to evaluate the relations between uterine size, total volume, and live birth outcomes, after they were adjusted for the main influencing factors. All \(P\) values corresponded to two-sided tests; \(P \leq 0.05\) was considered statistical significance. The variable assignment in the multivariate logistic regression analysis is shown in Table 1.

### 2.7. Ethical approval

The study was approved by the Ethics Committee of Xiangya Hospital of Central South University.

### 3. Results

#### 3.1. General information

Among the 8034 patients included in the study, 714 (8.9%) were lost to follow-up, and a total of 7320 women were included in this study. Live birth outcomes include live birth [5523 (75.5%)] and non-live birth [1797 (24.5%)]. Baseline clinical and demographic characteristics of the infertile Chinese Han women were shown in Table 2.

#### 3.2. Uterine length and live birth

We analyzed the relation between uterine length and live birth. In multivariate analysis, the determination of the influencing factors was through single factor analysis and through the multiple collinearity among influencing factors. The main influencing factors were number of previous ART treatments, Number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos and the quality of transferred embryos. Table 3 shows that compared with uterine lengths of 50 to 59 mm (referent), women with uterine lengths ≥ 60 mm had a lower live birth rate (RR = 1.541), and there was no significant difference in the live birth rate in women with uterine lengths 40 to 49 mm and <40 mm.

#### 3.3. Uterine width and live birth

Adjusted for number of previous ART treatments, number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos and the quality of transferred embryos, live birth was associated with uterine width (Table 4). Compared with women with uterine widths ≥50 mm (referent), women with uterine widths <30 mm had a lower live birth rate (RR = 1.430), and women with uterine widths of 30 to 39 mm and 40 to 49 mm did not have significantly different live birth rates.

#### 3.4. Uterine anteroposterior diameter and live birth

Table 5 shows the data for the relation between uterine anteroposterior diameter and live birth following clinical pregnancy in infertile Chinese Han women. In multivariate analysis, the determination of the influencing factors was through single factor analysis and through the multiple collinearity among influencing factors. The main influencing factors were number of previous ART treatments, total number of transferred embryos, and the quality of transferred embryos. Compared with uterine anteroposterior diameters of <30 mm (referent), women with uterine anteroposterior diameters ≥50 mm had a lower live birth rate (RR = 1.636), and women with uterine anteroposterior diameters 30 to 39 mm and 40 to 49 mm did not have significantly different live birth rates.

#### 3.5. Uterine volume and live birth

The relation between uterine volume and live birth outcome was statistically significant (Table 6). In multivariate analysis, the determination of the influencing factors was through single factor analysis and through the multiple collinearity among influencing factors. The main influencing factors were number of previous ART treatments, number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos, and the quality of transferred embryos. Compared with uterine volumes of 30 to 49 mL (referent), women with volumes <30 and ≥70 mL had lower live birth rates (RR = 1.368 and 1.742, respectively), and women with volumes 50 to 69 mL did not have significantly different live birth rate.

### 4. Discussion

The main new contribution of our study is the assessment of the relationship between uterine size, volume, and live birth outcome. The study has 4 major findings. First, women with uterine lengths
of ≥60 mm had a lower live birth rate. Second, women with uterine widths of <30 mm had a lower live birth rate. Third, the uterine anteroposterior diameters of ≥50 mm had a lower live birth rate. Finally, the uterine volumes of <30 or ≥70 mL had a lower live birth rate.

In our study, women with uterine lengths of ≥60 mm experienced a lower likelihood of live birth. The logistic regression demonstrates that the uterine length conferring the highest probability of live birth is closer to 50 mm. However, one study of uterine length and IVF outcomes in the United States reported that women with uterine lengths of >90 mm had a lower live birth rate, and women with uterine lengths closer to 80 mm had the highest probability of live birth.[14] Perhaps different races have their own reference uterine length range that is most

### Table 2

Demographic and clinical characteristics of 7320 infertile women (mean ± SD or N [%]).

| Live birth | Nonlive birth | P     |
|------------|--------------|-------|
| Age (years) 30.59 ± 4.37 | 31.06 ± 4.93 | <.001 |
| BMI (kg/m²) 21.61 ± 2.99 | 21.93 ± 3.11 | <.001 |
| Number of previous ART treatments 1.73 ± 0.98 | 1.82 ± 1.06 | <.001 |
| No. of antral follicles 12.89 ± 5.06 | 12.74 ± 6.13 | <.001 |
| Endometrial thickness before embryo transfer (mm) 10.52 ± 2.10 | 10.36 ± 2.10 | <.001 |
| Total no. of transferred embryos 1.98 ± 0.98 | 1.96 ± 0.33 | <.001 |
| Uterine length (mm) 50.33 ± 6.95 | 50.74 ± 7.49 | .020 |
| Uterine width (mm) 41.55 ± 7.21 | 41.38 ± 7.68 | .157 |
| Uterine anteroposterior diameter (mm) 45.95 ± 8.46 | 46.98 ± 6.75 | .017 |
| Uterine volume (mL) 31.53 ± 19.49 | 33.49 ± 23.79 | <.001 |

### Infertility diagnosis

| Live birth | Nonlive birth | P     |
|------------|--------------|-------|
| Male factor 742 (76.73) | 225 (23.27) | .414 |
| Ovulation dysfunction 28 (86.12) | 15 (34.88) | .831 |
| Decreased ovarian reserve 18 (64.29) | 10 (35.71) | .831 |
| Tubal factor 3406 (75.20) | 1123 (24.80) | .831 |
| Endometriosis 51 (71.83) | 20 (28.17) | .831 |
| Polycystic ovarian syndrome 175 (76.09) | 55 (23.91) | .831 |
| Chromosome abnormality 7 (50.00) | 7 (50.00) | .831 |
| Unexplained 20 (40.00) | 30 (60.00) | .831 |
| Male + female factors 1076 (77.52) | 312 (22.48) | .831 |

### Artificial insemination technologies

| Live birth | Nonlive birth | P     |
|------------|--------------|-------|
| IVF 4000 (75.23) | 1317 (24.77) | .831 |
| ICSI 1176 (76.96) | 352 (23.04) | .831 |
| IVF + ICSI 348 (76.63) | 106 (23.35) | .831 |
| IUI 18 (85.71) | 2 (14.29) | .831 |

### The quality of transferred embryos

| Live birth | Nonlive birth | P     |
|------------|--------------|-------|
| I 5043 (76.68) | 1534 (23.32) | .831 |
| II 311 (76.67) | 100 (24.33) | .831 |
| III 54 (74.56) | 40 (25.44) | .831 |

### Types of transferred embryos

| Live birth | Nonlive birth | P     |
|------------|--------------|-------|
| Fresh embryo 3240 (75.79) | 1035 (24.21) | .831 |
| Frozen–thawed embryo 2263 (74.93) | 757 (25.07) | .831 |

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of ≥60 mm had a lower live birth rate. Second, women with uterine widths of <30 mm had a lower live birth rate. Third, the uterine anteroposterior diameters of ≥50 mm had a lower live birth rate. Finally, the uterine volumes of <30 or ≥70 mL had a lower live birth rate.

In our study, women with uterine lengths of ≥60 mm experienced a lower likelihood of live birth. The logistic regression demonstrates that the uterine length conferring the highest probability of live birth is closer to 50 mm. However, one study of uterine length and IVF outcomes in the United States reported that women with uterine lengths of >90 mm had a lower live birth rate, and women with uterine lengths closer to 80 mm had the highest probability of live birth.[14] Perhaps different races have their own reference uterine length range that is most

### Table 3

Uterine length and live birth rate following ART in infertile Chinese Han women.

| Uterine length (mm) | No. | No. of live birth | Live birth rate (%) | RR (95% CI) | aRR (95% CI) |
|---------------------|-----|-------------------|---------------------|-------------|--------------|
| <40                 | 360 | 274               | 76.1                | 1.003 (0.777–1.296) | 1.210 (0.804–1.822) |
| 40~                 | 3023| 2290              | 75.8                | 1.023 (0.911–1.150) | 1.049 (0.866–1.271) |
| 50~                 | 3198| 2436              | 76.2                | 1 (referent)     | 1 (referent)   |
| ≥60                 | 739 | 523               | 70.8                | 1.320 (1.105–1.578) | 1.541 (1.161–2.047) |

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*RR calculated from univariate binary logistic regression.
*RR calculated from multivariate binary logistic regression adjusted for number of previous ART treatments, number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos and the quality of transferred embryos.
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Table 4

| Uterine width (mm) | No. | No. of live birth | Live birth rate (%) | RR (95% CI)† | aRR (95% CI)† |
|--------------------|-----|------------------|---------------------|--------------|--------------|
| <30                | 270 | 188              | 69.6                | 1.367 (1.016–1.839) | 1.430 (1.050–1.948) |
| 30~                | 2758| 2082             | 75.5                | 1.018 (0.860–1.204) | 1.082 (0.909–1.288) |
| 40~                | 3279| 2485             | 75.8                | 1.002 (0.850–1.181) | 1.040 (0.880–1.230) |
| ≥50                | 1013| 768              | 75.8                | 1 (referent)     | 1 (referent)   |

† RR calculated from univariate binary logistic regression.

RR calculated from multivariate binary logistic regression adjusted for number of previous ART treatments, number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos and the quality of transferred embryos.

Table 5

| Uterine anteroposterior diameter (mm) | No. | No. of live birth | Live birth rate (%) | RR (95% CI)† | aRR (95% CI)† |
|---------------------------------------|-----|------------------|---------------------|--------------|--------------|
| <30                                   | 115 | 94               | 81.7                | 1 (referent) | 1 (referent) |
| 30~                                   | 1503| 1153             | 76.7                | 1.359 (0.834–2.213) | 1.426 (0.870–2.338) |
| 40~                                   | 3242| 2470             | 76.2                | 1.399 (0.866–2.261) | 1.444 (0.887–2.351) |
| ≥50                                   | 2460| 1806             | 73.4                | 1.621 (1.002–2.623) | 1.636 (1.010–2.648) |

† RR calculated from univariate binary logistic regression.

Table 6

| Uterine volume (mL) | No. | No. of live birth | Live birth rate (%) | RR (95% CI)† | aRR (95% CI)† |
|---------------------|-----|------------------|---------------------|--------------|--------------|
| <30                 | 690 | 504              | 73.0                | 1.229 (1.019–1.482) | 1.368 (1.003–1.864) |
| 30~                 | 3191| 2454             | 76.9                | 1 (referent)     | 1 (referent)   |
| 50~                 | 2325| 1778             | 76.5                | 1.024 (0.903–1.162) | 1.161 (0.942–1.431) |
| ≥70                 | 1114| 787              | 70.6                | 1.384 (1.187–1.612) | 1.742 (1.360–2.232) |

† RR calculated from univariate binary logistic regression.

RR calculated from multivariate binary logistic regression adjusted for number of previous ART treatments, number of antral follicles, stimulation protocol, endometrial thickness before embryo transfer, total number of transferred embryos and the quality of transferred embryos.

Table 4

Uterine width and live birth rate following ART in infertile Chinese Han women.

Table 5

Uterine anteroposterior diameter and live birth rate following ART in infertile Chinese Han women.

Table 6

Uterine volume and live birth rate following ART in infertile Chinese Han women.

suitable for the growth and development of the foetus. Of course, other factors have not been excluded, such as different methods of measurement.

Women with uterine widths of <30 mm or anteroposterior diameters of ≥50 mm experienced a lower likelihood of live birth, women with uterine widths closer to 50 mm and uterine anteroposterior diameters closer to 30 mm had the highest probability of live birth. Until now, no research has reported these probabilities of live birth for uterine widths and diameters.

The women with uterine volumes of <30 or ≥70 mL experienced a lower likelihood of live birth; women with a uterine volume closer to 50 mL had the highest probability of live birth. Previous studies focused on uterine volume related to non-cavity-distorting and cavity-distorting uterine fibroids and live birth rate.[20–22] The relation between uterine volume and live birth rate following ART has not been published.

The potential mechanisms for a lower likelihood of live birth in women at the extremes of uterine size and volume may be multiple and complicated, including anatomical, hormonal, and genetic explanations. Though women with perceptible congenital abnormal uterus were excluded, extremes of uterine size and volume may signify anatomical variations that contribute to a decreased likelihood of implantation.[14] Some studies documented a positive association between uterine size and estrone concentration, which implied that the extremes of uterine size might reflect estrone deficiency or excess that adversely impacts on ART success,[14,23,24] abnormal production or action of GH, progesterone and IGFs might affect it too.[23,26] Furthermore, uterine size was influenced by the congenital abnormalities in HOX and Wnt gene expression.[27]

The strength of our study first revealed their inherent relations between uterine size, volume and live birth outcome following ART in infertile women in Asia. These findings are more objective and credible because of sufficient sample size, accurate measurement, and adjustment for known confounders. These data may provide a reference for gynaecologists in the diagnosis and treatment of infertility.

The main limitation of the study is that we only studied infertile Chinese Han women and did not include a control group. The generalizability of our findings to other races and to the normal population remains unclear.

In conclusion, there were significant correlations between uterine size, volume, and live birth outcomes following ART in infertile women. The extremes of uterine lengths, widths, and volumes had the lowest live birth rate. Uterine size and volume that were too large or too small reduced live birth rates. Our findings may stimulate further research on the establishment of a prediction model based on uterine size and volume.
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