

# Fecundity disorders in older women: declines in follicular development and endometrial receptivity

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## Research article

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# Abstract

**Background** Fecundity declines in older women are associated with decreased ovarian function and oocyte quality, but little research is available on endometrial receptivity in older women. This study aimed to assess follicular development and endometrial receptivity, and to evaluate ultrasonic parameters in predicting endometrial receptivity during the implantation window in older women.

**Methods** For this prospective case-control study, 224 older women and 215 women under the age of 35 were recruited. The follicular development and endometrial thickness were monitored by transvaginal ultrasound before ovulation. During the implantation window, defined as 6 to 7 days after ovulation, the pulsatility index (PI) and resistance index (RI) of the uterine arteries and subendometrial region were calculated based on colour Doppler, and the endometrial volume, vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were also determined using the VOCAL imaging program with 3-D mode power Doppler. The ultrasonic parameters were used to assess endometrial receptivity in older women.

**Results** The serum anti-Mullerian hormone (AMH) concentration and antral follicle count (AFC) were significantly lower in older women than in controls ( $P < 0.05$ ). The average diameter of the dominant follicle was significantly smaller, and the subendometrial region RI was significantly higher in older women compared with controls ( $P < 0.05$ ). The normal ovulation rate was significantly lower in older women than in controls ( $P < 0.01$ ). The subendometrial region RI was significantly higher, and the endometrial VI, FI, and VFI were significantly lower in older women compared with controls ( $P < 0.05$ ). The biochemical pregnancy rate, clinical pregnancy rate and ongoing pregnancy rate of older women were significantly lower than those of controls ( $P < 0.05$ ). The best ultrasonic parameter for predicting endometrial receptivity during the implantation window in older women was VI, followed by FI.

**Conclusions** Older women present decreased serum AMH concentrations and AFC, defined as indicators of ovarian reserve function. Older women are characterized by decreased follicular development and endometrial receptivity, which may lead to fecundity disorders.

## Background

In recent years, later-age childbearing has become a trend in China, particularly as more women have steadily entered the workforce. In addition, with the implementation of the two-child policy in China, the number of older women ( $\geq 35$  years) who desire pregnancy is gradually increasing. However, fecundity declines significantly beginning at approximately 32 years of age and decreases more rapidly after 37 years of age because of a decrease in egg quality and a gradual increase in the circulating level of follicle-stimulating hormone (FSH) [1]. Therefore, many older women will face fecundity disorders, including decreased egg quality, pregnancy rate and live birth rate, as well as increased embryonic aneuploidy and abortion rate [2].

It is well known that fecundity declines in older women are associated with decreased ovarian function and oocyte quality. However, little research is available on endometrial receptivity in older women. Endometrial receptivity plays a crucial role in the implantation of the embryo, and its impairment has been shown to be one of the factors contributing to infertility in women. Transvaginal ultrasound is the first-line method to observe follicular development and endometrial growth because of its non-invasive characteristics, and ultrasonic parameters, including endometrial thickness, classification and blood flow, have been confirmed to reflect endometrial receptivity [3–4]. This study aimed to assess ovarian reserve function, follicular development and endometrial receptivity in older women and to evaluate the predictive value for endometrial receptivity of different ultrasonic parameters during the implantation window.

## Materials And Methods

### Study design and participants

This prospective case-control study was conducted in the First Affiliated Hospital of Xi'an Jiaotong University from January 2017 to April 2019. A total of 224 older women ( $\geq 35$  years) and 215 women under the age of 35 who wanted to become pregnant were recruited. Women were included if they had regular menstrual cycles (21-35 days), bilateral fallopian tubal patency detected by hysterosalpingogram, and partners with normal semen according to WHO criteria [5]. Women were excluded if they had polycystic ovarian syndrome, thyroid disease, gynaecological surgery, pelvic inflammatory diseases or endometriosis. Socio-demographic information such as age, body mass index (BMI), number of gravidity and deliveries, and menstrual cycle were obtained by a questionnaire. Sex hormone and anti-Mullerian hormone (AMH) concentrations were detected in our hospital laboratory. The baseline antral follicle count (AFC) was determined, which was defined as the total number of antral follicles (follicles measuring 2–10 mm) in both ovaries. All participants gave their informed consent for inclusion before they participated in the study. The protocol was approved by the First Affiliated Hospital of Xi'an Jiaotong University Institutional Review Board.

### Outcome measures

The follicular development, endometrial thickness (ET), pulsatility index (PI) and resistance index (RI) of the uterine arteries and subendometrial region were monitored by transvaginal ultrasound every two days before ovulation. Timed intercourse was advised when the dominant follicle reached 18 mm in diameter. In addition, during the implantation window, defined as 6 to 7 days after ovulation, the endometrial volume, vascularization index (VI), flow index (FI) and vascularization flow index (VFI) were determined using the virtual organ computer-aided analysis (VOCAL) imaging program with 3-D mode power Doppler. All ultrasonic parameters were measured three times, and the average value was used for the final statistical analysis. Luteinized unruptured follicular syndrome (LUFS) was defined as persistent existence or enlargement of follicles after maturation, thickening of follicle walls, and a strength echo in the point or grid shape or a cystic solid echo with great tension inside the follicle.

Pregnancy rates were observed during this menstrual cycle, including biochemical pregnancy (defined as human chorionic gonadotropin level  $\geq 10$  mIU/mL measured at 2 weeks after ovulation), clinical pregnancy (defined as the presence of embryo in the uterine cavity) and ongoing pregnancy (confirmed as the presence of a foetus with heart motion at 12 weeks of gestation) [6].

The primary outcome included follicular development and ultrasonic parameters of endometrial receptivity. The secondary outcomes were the pregnancy rates and the predictive value of different ultrasonic parameters for endometrial receptivity in older women.

## Statistical analysis

The data in this study were statistically analyzed by SPSS version 16.0. The descriptive statistics for continuous variables were given as mean  $\pm$  SD and analyzed by Student's t-test. Differences in dichotomous outcomes were compared by chi-square test. The evaluation of endometrial receptivity of different parameters was done by receiver operating characteristic (ROC) curves.  $P < 0.05$  was considered statistically significant.

## Results

The data in Table 1 suggest that the serum AMH concentration and AFC were significantly lower in older women than in controls ( $P < 0.05$ ). No significant difference was found when comparing other basic clinical data between the two groups ( $P > 0.05$ ).

Table 1 Clinical data between older women and controls.

Characteristics	Older women (n=224)	Controls (n=215)	<i>P</i> value <sup>a</sup>
BMI (kg/m <sup>2</sup> )	21.46 $\pm$ 5.45	22.09 $\pm$ 4.87	0.658
Number of gravidity	2.17 $\pm$ 0.86	2.35 $\pm$ 0.90	0.437
Number of delivery	0.97 $\pm$ 0.42	0.41 $\pm$ 0.16	0.039
Menstrual cycle (days)	30.12 $\pm$ 5.53	31.45 $\pm$ 6.02	0.225
Basic concentrations			
FSH (mIU/mL)	7.80 $\pm$ 2.76	6.35 $\pm$ 2.01	0.367
LH (mIU/mL)	5.53 $\pm$ 1.12	5.48 $\pm$ 1.29	0.216
PRL (ng/mL)	10.14 $\pm$ 3.37	8.45 $\pm$ 2.48	0.103
E <sub>2</sub> (pmol/L)	91.35 $\pm$ 25.63	102.47 $\pm$ 30.42	0.098
P (nmol/L)	1.09 $\pm$ 0.76	1.17 $\pm$ 0.90	0.342
T (nmol/L)	0.92 $\pm$ 0.41	0.86 $\pm$ 0.44	0.677
AMH (ng/mL)	1.02 $\pm$ 0.58	2.65 $\pm$ 0.99	0.019
AFC (number)	8.53 $\pm$ 2.21	15.67 $\pm$ 4.32	0.022

Data given as mean  $\pm$  SD. <sup>a</sup> T-test.

FSH: follicle stimulating hormone, LH: luteinizing hormone, PRL: prolactin, E<sub>2</sub>: estradiol, P: progesterone, T: testosterone.

Figure 1 indicates the follicular development and the PI/RI of the uterine arteries and subendometrial region before ovulation. The average diameter of the dominant follicle on days 14, 16, and 18 of the menstrual cycle were significantly smaller, and the subendometrial region RI on days 12, 14, 16, and 18 of the menstrual cycle were significantly higher in older women than in controls ( $P < 0.05$ ) (Fig. 1A and F). However, no significant difference was found when comparing endometrial thickness, average uterine PI and RI, and subendometrial region PI between the two groups ( $P > 0.05$ ) (Fig. 1B-E).

Figure 2 reveals that the normal ovulation rate in older women was significantly lower compared with controls [69.64% (156/224) vs. 82.79% (178/215),  $P < 0.01$ ], and the without dominant follicle rate in older women was significantly higher than that in controls [21.88% (49/224) vs. 9.77% (21/215),  $P < 0.01$ ]. However, no significant difference was found when comparing the LUFs rate between the two groups [8.48% (22/224) vs. 7.44% (16/215),  $P > 0.05$ ].

Table 2 shows the ultrasonic parameters of endometrial receptivity during the implantation window in women with normal ovulation between the two groups. The subendometrial region RI was significantly higher, and the endometrial VI, FI, and VFI were significantly lower in older women than in controls ( $P < 0.05$ ). The data in Table 3 indicate that the biochemical pregnancy rate, clinical pregnancy rate and ongoing pregnancy rate of older women were significantly lower than those of controls ( $P < 0.05$ ).

Table 2. Ultrasonic parameters of endometrial receptivity during implantation window in women with normal ovulation between the two groups.

Parameters	Older women (n=156)	Controls (n=178)	<i>P</i> value <sup>a</sup>
Average uterine PI	2.03±0.74	2.08±0.81	0.897
Average uterine RI	0.76±0.13	0.75±0.15	0.903
Subendometrial region PI	0.98±0.24	1.00±0.29	0.890
Subendometrial region RI	0.70±0.12	0.62±0.11	0.041
Endometrial thickness (mm)	9.01±2.41	9.67±2.68	0.335
Endometrial volume (cm <sup>3</sup> )	2.93±0.87	3.24±1.02	0.126
Endometrial VI (%)	1.72±0.92	3.04±1.13	0.019
Endometrial FI (0-100)	19.5±4.65	32.3±6.32	0.015
Endometrial VFI (0-100)	0.33±0.02	0.97±0.06	0.007

Data given as mean ± SD.

<sup>a</sup>T-test.

Figure 3 shows that the best ultrasonic parameter for predicting endometrial receptivity during the implantation window in older women was VI (AUC=0.889, sensitivity 92.6% and specificity 85.4%), followed by FI (AUC=0.838, sensitivity 90.7% and specificity 82.1%).

## Discussion

Although there are several indicators to evaluate ovarian function, age is the primary determinant of reproductive potential. Data suggest that among populations that do not use contraception, fertility rates decrease with increasing age of women [7]. The cumulative pregnancy rate observed across up to 12 insemination cycles was 74% for women younger than 31 years, decreased to 62% for women aged 31–35 years and decreased further to 54% for women older than 35 years [1]. Therefore, the pregnancy success rate of older women is significantly reduced in regard to both natural conception and assisted reproductive technology [8]. According to reports in the literature, pregnancy loss is closely related to age. Whereas 9.9% of women younger than 33 years who conceive during IVF with a fresh embryo transfer experience pregnancy loss after foetal heart activity is observed, the rate of miscarriage progressively increases to 13.7% for women aged 35–37 years and to 19.8% for women aged 38–40 years [9]. Our findings show that the biochemical pregnancy rate, clinical pregnancy rate and ongoing pregnancy rate in older women were significantly lower than those in controls.

Several indicators can be used to assess ovarian reserve function, including FSH, inhibin B, AMH and AFC. The data in this study revealed that AMH and AFC were superior to menstrual cycles and FSH in evaluating ovarian function in older women, which is consistent with the results reported in most previous research [10–11]. As a woman ages, her oocyte and follicular pool declines, so AFC is a good indicator of ovarian function in women. As the oocyte and follicular pool declines, granulosa cells secrete less.

Although the ability of AMH to predict reproductive potential is controversial, it is an excellent predictor of oocyte yield among women with infertility undergoing controlled ovarian hyperstimulation for in vitro fertilization (IVF) [12]. In addition, data from our study showed that the average diameter of the dominant follicle in older women was significantly smaller, and the normal ovulation rate was significantly lower in older women, which is associated with the fecundity declines of older women.

Embryo implantation is a complicated process in which the blastocyst interacts with the receptive endometrium. In the normal reproductive cycle of humans and mammals, there is a very short period during which the endometrium is receptive for embryo implantation, which is defined as 6 to 7 days after ovulation in the normal menstrual cycle. In the early stage of embryo implantation, angiogenesis is active, and the expression of various angiogenesis-related factors is increased, which provides support for embryonic development and pregnancy. Therefore, the blood supply of the endometrium is of great significance to its receptivity. Our previous results showed that transvaginal two-dimensional ultrasound could evaluate endometrial receptivity by detecting endometrial thickness and blood flow [13].

At present, three-dimensional energy Doppler ultrasound, which can reflect the vascular configuration of organ tissues and the richness of blood flow, has been used to assess endometrial receptivity [14]. Studies have confirmed that the value of this technique in evaluating endometrial receptivity is better than that of two-dimensional ultrasound, and it has been reported to be used to evaluate endometrial receptivity for in vitro fertilization-embryo transfer (IVF-ET) [15]. The data in our study showed that the subendometrial region RI was significantly higher and that the endometrial VI, FI, and VFI during implantation window were significantly lower in older women than in controls, which might be related to the decreased endometrial receptivity. Furthermore, the data in this study show that the best ultrasonic parameter for predicting endometrial receptivity during the implantation window in older women was VI, followed by FI. Wang et al. showed that increased endometrial blood flow in IVF-ET infertile women during follicular maturation was beneficial to pregnancy [16]. Other studies have found that three-dimensional energy Doppler ultrasonography can assess endometrial receptivity and predict pregnancy outcome by detecting follicular maturation day and embryo transfer day with intrauterine and subintimal blood flow [17].

This study has some limitations. Fecundity disorders in older women are associated with multiple factors, including follicular dysplasia, increased aneuploidy and decreased endometrial receptivity. In addition, as age increases, the risks of reproductive diseases that may adversely affect fertility, such as fibroids, tubal disease, and endometriosis, also increase. Furthermore, this was a prospective case-control study in a single centre, and the sample size was relatively small. Therefore, the repeatability of the results of this study needs to be confirmed by multi-centre surveys with large sample sizes.

## Conclusions

Older women present decreased ovarian reserve function, for which the predictive value of AMH and AFC is more sensitive. Older women present decreased follicular development and endometrial receptivity,

which might be related to fecundity disorders. The results of this study provide new ideas for the improvement of pregnancy rate and reproductive outcomes in older women.

## **Abbreviations**

BMI: body mass index; AMH: anti-Mullerian hormone; AFC: antral follicle count; LUFS: luteinized unruptured follicular syndrome; FSH: follicle stimulating hormone; LH: luteinizing hormone; PRL: prolactin; E<sub>2</sub>: estradiol; P: progesterone; T: testosterone; PI: pulsatility index; RI: resistance index; ET: endometrial thickness; EV: endometrial volume; VI: vascularization index; FI: Flow index; VFI: vascularization flow index.

## **Declarations**

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### **Authors' contributions**

Study design: LW and SL. Methodology: WJ. Investigation: EB. Writing-review and editing: XF. All authors read and approved the final manuscript.

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### **Ethics approval and consent to participate**

The First Affiliated Hospital of Xi'an Jiaotong University Institutional Review Board and all participants gave their informed consent for inclusion before they participated in the study.

### **Availability of data and materials**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

### **Author details**

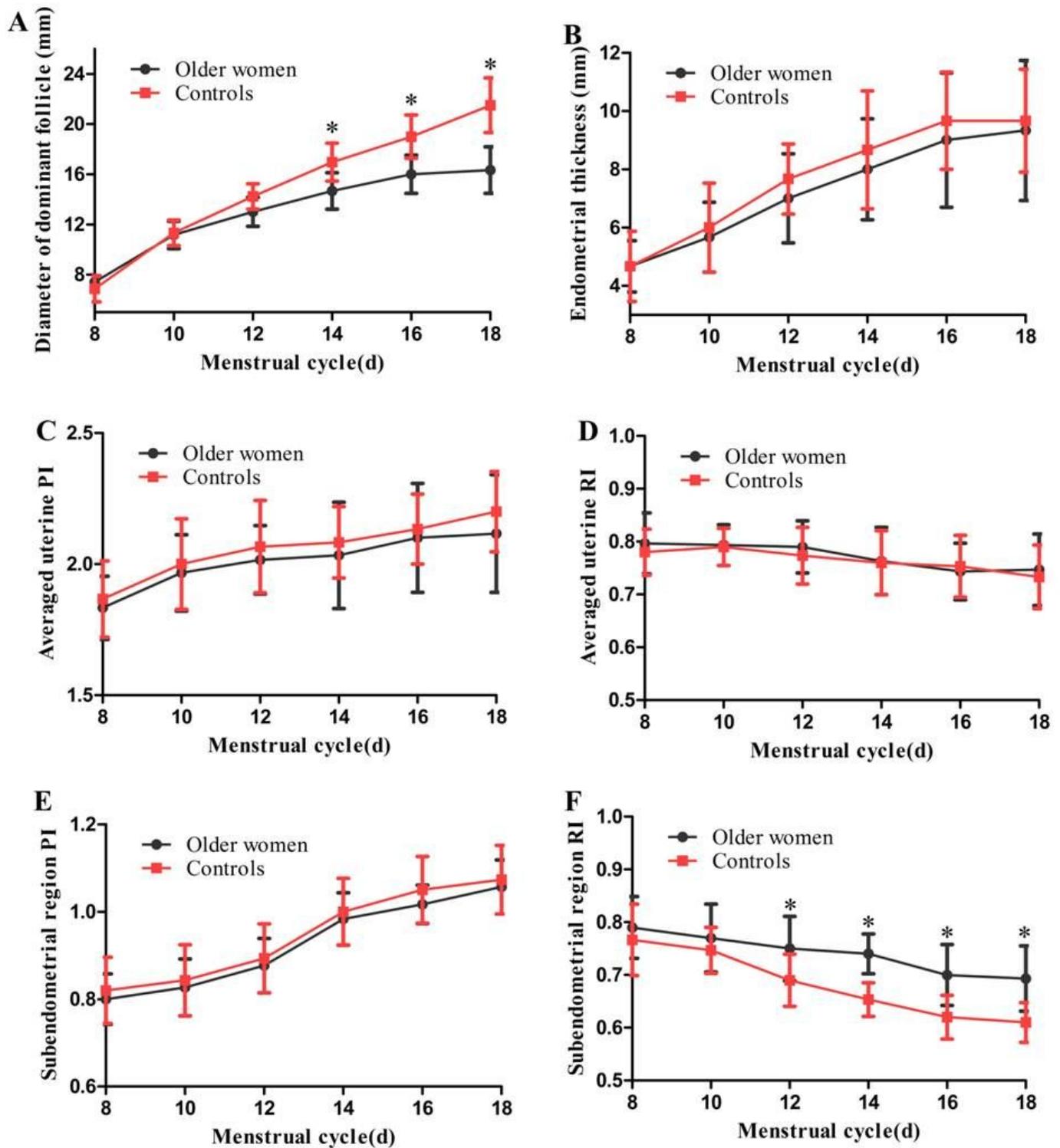
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## Figures



**Figure 1**

Follicular development, PI/RI of the uterine arteries and subendometrial region before ovulation between older women and controls (PI: pulsatility index, RI: resistance index, \* $P < 0.05$ ).

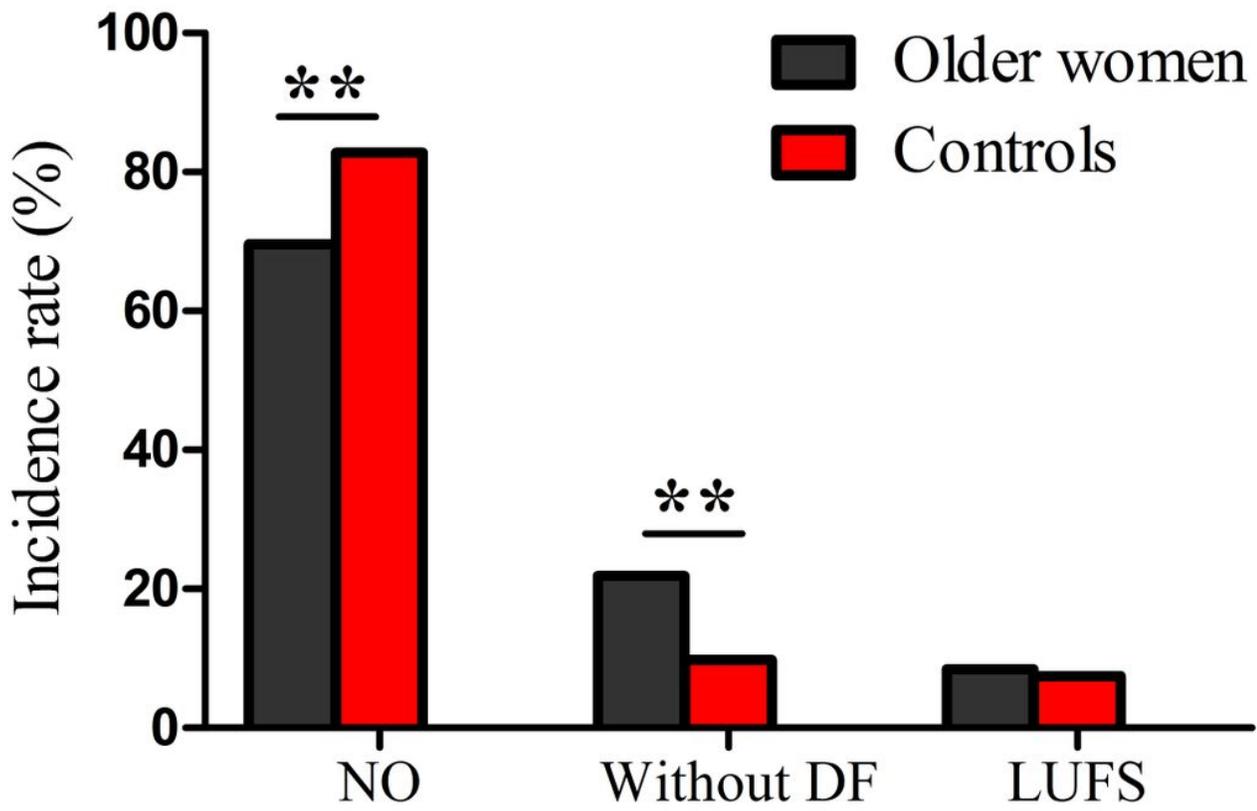
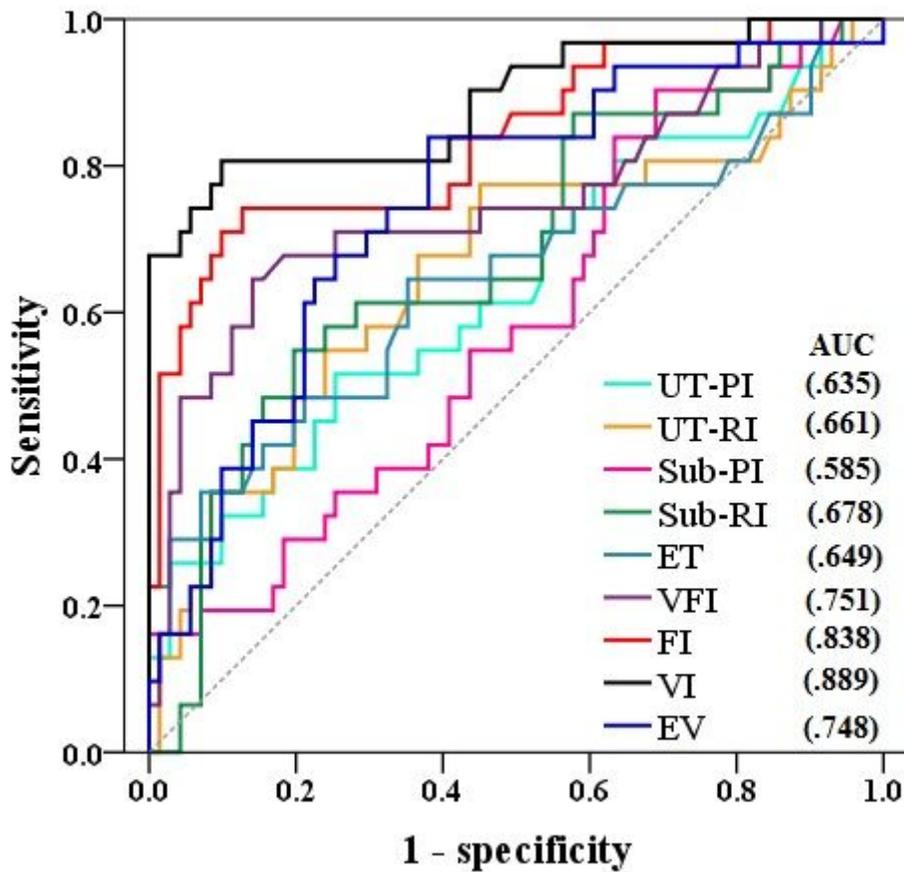


Figure 2

Follicular development and ovulation rate between older women and controls (NO: Normal ovulation, DF: Dominant follicle, LUFS: Luteinized unruptured follicular syndrome)



**Figure 3**

ROC curves of the predictive value for endometrial receptivity during implantation window in older women (UT-PI: uterine artery pulsatility index, UT-RI: uterine artery resistance index, Sub-PI: subendometrial region pulsatility index, Sub-RI: subendometrial region resistance index, ET: endometrial thickness, EV: endometrial volume, VI: vascularization index, FI: Flow index, VFI: vascularization flow index).