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# Ovarian volume and antral follicle count assessed by MRI and transvaginal ultrasonography: a methodological study

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**Declaration of interest:** The authors have nothing to disclose.

**Running Title:** Imaging of ovarian morphology

## ABSTRACT

**Background:** Ultrasonographic measurements of ovarian volume and antral follicle count are of clinical importance as diagnostic features of polycystic ovarian syndrome (PCOS), and as a parameter in estimation of ovarian follicular reserve in infertility care.

**Purpose:** To compare two-dimensional (2D)/three-dimensional (3D) TVUS and MRI for estimation of ovarian volume and antral follicle count, and assess reproducibility and interobserver agreement of MRI measurements.

**Material and Methods:** Volumes of 172 ovaries in 99 women aged 21-37 years were calculated ( $length \times width \times height \times 0.523$ ) with conventional 2D-TVUS and 2D-MRI. Semi-automatic estimates of ovarian volumes were obtained by 3D-MRI. Antral follicles were counted manually on 2D-MRI and automatically by 3D-TVUS (SonoAVC), and stratified according to follicle size.

**Results:** Mean ovarian volume assessed by 2D-TVUS ( $13.1 \pm 6.4$  ml) was larger than assessed by 2D-MRI ( $9.6 \pm 4.1$ ) and 3D-MRI ( $11.4 \pm 4.5$ ) ( $P < 0.001$ ). Total follicle count was higher by 2D-MRI than by 3D-TVUS, mean difference  $14.3 \pm 16.2$  follicles ( $P < 0.001$ ). In the smallest size interval of 1-3 mm the mean difference was  $22.2 \pm 17.6$  ( $P < 0.001$ ). Intra- and interobserver absolute agreement assessment for MRI measurements of ovarian volume and total follicle count showed ICC coefficients exceeding 0.77.

**Conclusion:** 2D-MRI reveals more antral follicles, especially of small size, than 3D-TVUS. Ovarian volume estimation by MRI provides smaller volumes than by the reference standard 2D TVUS. Ovarian volume estimation by 3D-MRI, allowing independence of non-ellipsoid ovarian shape measurement errors, provides volumes closer to 2D-TVUS values than does 2D-MRI. Reproducibility and interobserver agreement of 2D-MRI measurements of ovarian volume and total follicle count are good.

**Keywords:** ovarian morphology; ovarian volume; antral follicle count; magnetic resonance imaging; ultrasonography; three-dimensional imaging

In clinical practice, transvaginal ultrasonography (TVUS) is the first choice imaging modality in evaluating the ovaries because of its high performance, availability, cost-effectiveness, and patient friendliness. The major limitations of TVUS are its user dependency and the limitations in displaying a global view of the pelvis and large lesions of ovarian origin. Magnetic resonance imaging (MRI), with its excellent soft-tissue contrast resolution and characteristics, is a useful non-invasive alternative modality to TVUS, especially in adolescent and/or very obese women (1, 2) or when TVUS findings are indeterminate (3). There are numerous reports on MRI performance in evaluating adnexal masses, but reports on MRI in normal (4, 5) or close to normal appearing ovaries, such as polycystic ovaries (PCO), are quite rare (1, 6-8). Both modalities have undergone an impressive technical development during the last decade, including the introduction of three-dimensional (3D) techniques. Automated 3D TVUS technique has been found to be significantly quicker in estimating the number of ovarian antral follicles, but fewer follicles may be observed as compared to the reference standard two-dimensional (2D) TVUS (9).

**The cortex occupies the greater part of the ovary and its stroma of primitive connective tissue contains the follicles; primordial, primary, secondary and tertiary (or antral, from the term *antrum* meaning a cavity or chamber, here fluid filled; cystic) follicles. Only the fluid-containing antral follicles can be distinguished by TVUS or MRI. They are recognized as thin-walled fluid containing structures in the ovary, hypoechoic on TVUS, and with homogeneously high signal intensity on T2-weighted MRI sequences and low signal intensity on T1-weighted sequences.**

Measurements of ovarian volume and antral follicle count are of clinical importance as diagnostic features of polycystic ovarian syndrome (PCOS), and as a parameter in estimation of ovarian follicular reserve for prognostic purposes in infertility care (1, 9-12). **A subfertile woman's age alone can not predict the ovarian response to assisted reproductive**

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**therapy. Antral follicle count contributes to predict and individualize treatment strategies, such as in vitro fertilization (11). For diagnosis of PCOS, the 2003 Rotterdam criteria require at least two of the following: oligomenorrhea or anovulation, clinical or biochemical signs of hyperandrogenism, and at least one polycystic ovary defined as the presence of  $\geq 12$  follicles 2–9 mm in diameter or ovarian volume  $>10$  ml at ultrasonography (12). However, recent advancements in imaging technology has questioned the accuracy of these morphologic criteria, and higher thresholds of antral follicle counts have been proposed (13, 14).** There is a need to evaluate performance of modern techniques on ovarian imaging, for clinical as well as scientific purposes.

The objective of the present study was to compare 2D/3D TVUS and 2D/3D MRI for estimation of ovarian volume and number and size distribution of ovarian antral follicles in adult women of reproductive age, who were examined with both techniques, and to assess the intraobserver and interobserver agreement of MRI measurements.

## **Material and Methods**

### *Study population and study design*

This prospective cross-sectional study was conducted on women with and without polycystic ovary syndrome (PCOS) from November 2005 to September 2008 at the Sahlgrenska University Hospital, performed in accordance with the Declaration of Helsinki, and approved by the Regional Ethical Review Board. All participants gave oral and written consent. Clinical and demographic characteristics on the study population, and MRI data not related to the present purpose, have previously been reported (15, 16).

Potential participants were recruited by advertising in the local community. They were asked to describe their medical history and those included underwent a gynecological

examination including a screening TVUS followed by 2D/3D TVUS and MRI as described below.

#### *Transvaginal ultrasonography*

The 2D and 3D TVUS examinations were performed using a Voluson Expert 730™ (GE Healthcare, Zipf, Austria) ultrasound machine with a multi-Hz vaginal transducer. Examinations were performed by a senior ultrasound specialist (gynaecologist B.G.).

The 2D TVUS ovarian volume was calculated as ovarian *length x width x height x 0.523*.

The number of follicles in the size intervals 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21, and  $\geq 22$  mm were counted by 3D technique, using the software program SonoAVC (Sono-Automatic Volume Calculation, GE Healthcare). Within a 3D data set, hypoechoic regions are identified and provide automatic estimations of their absolute dimensions. Each defined volume interval is given a specific color and the diameters are displayed (Fig. 1). In this initial automatic assessment follicles may be missed, but such follicles can be included in the analysis by manually clicking on them. SonoAVC has previously been reported to be useful for follicle tracking and estimation of antral follicle count (9, 17).

#### *Magnetic resonance imaging*

MRI was performed immediately after the 2D/3D TVUS examinations, in a 1.5 Tesla scanner (Intera; Philips Medical Systems, Best, The Netherlands), with a pelvic phased-array coil for signal reception. For ovarian morphologic imaging, pelvic multislice T2-weighted turbo spin-echo (TSE) acquisitions were obtained in transaxial (repetition time/echo time 3700-4500/120 msec, flip angle 90°, field of view 230 mm, slice thickness 4 mm, gap 1 mm, matrix 352 x 352), sagittal, and coronal planes.

MRI data were transferred to a workstation (Centricity Workstation Radiology RA 600; GE Healthcare, Little Chalfont, Buckinghamshire, UK). Ovarian morphology was evaluated by an experienced radiologist (Observer 1; H.L.), blinded to the TVUS findings. All visible follicles, defined as thin-walled fluid containing structures in the ovary with homogeneously high signal intensity on T2-weighted images, were manually counted in at least two orthogonal planes. Follicles were categorized according to size, using the size intervals 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21, and  $\geq 22$  mm. The size of an individual follicle was estimated as the mean of the two largest orthogonal diameters in one plane. Ovarian volume was calculated by the standard ellipsoid formula *ovarian length x width x height x 0.523*, using electronic calipers to mark the largest diameters on images obtained in orthogonal planes (Fig. 2).

MRI data were also transferred to a workstation (Advantage Windows Analysis Station; GE Healthcare) allowing semi-automatic volume estimates. The peripheral border of the ovary was outlined by a caliper in the transaxial plane in each slice containing ovarian tissue. By use of the workstation's semi-automatic software utilizing a seeding procedure, total ovarian volume was then obtained (Fig. 3). These 3D data were obtained by a medical student, after initial training and subsequent supervision from the experienced radiologist for quality control.

For test of intraobserver agreement, the MRI images of 30 ovaries in 15 randomly selected women (10 PCOS, 5 controls) were re-evaluated by Observer 1 after one year in order to avoid recall-bias, blinded to the first set of measurements. For estimation of interobserver agreement, 30 ovaries in 15 randomly selected MRI examinations were independently and blindly evaluated by a second radiologist (senior resident; Observer 2), with limited previous experience of gynecologic MRI. Ovarian volume estimation, using the formula *ovarian length x width x height x 0.523*, and antral follicle counting were performed.

### *Statistical methods*

The data are presented as median with range, and as mean or mean differences with standard deviation (SD). The variables were not normally distributed and differences between MRI and TVUS measurement were determined by Wilcoxon signed ranks test. Correlation coefficients were assessed by Spearman's rho. Intraobserver and interobserver agreements were estimated by intraclass correlation coefficients (ICC) [2, 1], which is a two-way random single measures analysis of variance (ANOVA) model with absolute agreement (18). An ICC coefficient of 0.5 usually represents a moderate agreement, a value of 0.7 is considered a good agreement and above 0.8 a very good agreement. Mean differences between measurements and 95% (2 x SD) intervals on either side of the mean as limits of agreement, were calculated for comparisons and presented as Bland-Altman plots.  $P < 0.05$  was considered significant. Statistical analyses were conducted with PASW Statistics (version 18.0 for Windows; SPSS Inc, Chicago, IL).

### **Results**

Ninety-nine women were included. In each subject two ovaries were visualized by MRI (100%). **With 2D/3D TVUS, the corresponding figure was 98%** (two ovaries on the left side, and one ovary on the right side, were not visualized by 2D/3D TVUS). For technical reasons, 2D TVUS data were not available for a further 9 ovaries. Thus, in total 186 ovaries were available for comparison of ovarian volume estimation by 2D TVUS and 2D/3D MRI. However, in 14 ovaries, 20 mm or larger partly exophytic cysts were found, and these non-ellipsoid ovaries were excluded from calculation of ovarian volume, leaving 172 ovaries for volume analyses. For technical reasons 3D TVUS data were missing in 26 ovaries. Thus, in

total 169 ovaries were available for comparison of antral follicle counts by 3D TVUS versus 2D MRI.

Apart from a hemorrhagic cyst/endometrioma of less than 15 mm in size in the right ovary of two women, detected by MRI, no ovarian pathology other than PCO was found.

#### *Ovarian volume*

The ovarian volumes measured by the different methods are presented in Table 1. The mean ovarian volume assessed by the reference standard 2D TVUS ( $13.1 \pm 6.4$  ml) was larger than assessed by 2D MRI ( $9.6 \pm 4.1$  ml) and 3D MRI ( $11.4 \pm 4.5$  ml) ( $P < 0.001$ ). The correlation between 2D TVUS - 2D MRI and 2D TVUS - 3D MRI was high ( $r=0.70$  and  $r=0.75$  respectively), and very high between 2D MRI - 3D MRI ( $r=0.87$ ) (Table 2).

The distributions of the differences are illustrated by Bland-Altman plots (Fig. 4-6). A larger deviation was noted in the larger volume estimations.

#### *Antral follicle count*

The antral follicle counts assessed by the different methods are presented in Table 3. The total follicle count was higher by 2D MRI (median 33, range 12 - 102 follicles) than by 3D TVUS (median 22, range 3 - 110), with a mean difference of  $14.3 \pm 16.2$  ( $P < 0.001$ ) follicles. The most prominent difference **in number of follicles** between 2D MRI and 3D TVUS was observed in the smallest size interval of 1-3 mm with a mean difference of  $22.2 \pm 17.6$  ( $P < 0.001$ ) follicles, although all size intervals differed between the methods except for follicle size  $\geq 19$  mm (Table 3). There was a correlation between the two methods in all size intervals except for follicle size  $\geq 19$  mm, although with a low correlation coefficient (Table 3). The

total follicle count and number of follicular cysts 22 mm or larger for 2D MRI and 3D TVUS was strongly correlated (Table 3).

#### *Reproducibility of MRI measurements and agreement between different observers*

The intraobserver agreement for MRI measurements of ovarian volume and follicle count was very good, with ICC coefficients exceeding 0.86 apart from in size interval 7-9 mm, as summarized in Table 4. The interobserver agreement for MRI measurements of ovarian volume was very good and for total follicle count good, but inconsistent in the smaller size intervals, as presented in Table 5.

#### **Discussion**

To our knowledge, this is the first study of direct comparison of MRI and TVUS in evaluation of ovarian volume and antral follicle count. In an earlier study of 11 obese adolescents with PCOS, imaging results of MRI were compared with *transabdominal* ultrasonography (2). With MRI, the mean total follicle count (21.9) was greater than that observed by transabdominal ultrasonography (5.5). Estimations of ovarian volumes were not compared in the adolescents, but the mean cross-sectional total ovarian area was greater as assessed by ultrasonography (619 mm<sup>2</sup>) than by MRI (487 mm<sup>2</sup>). Other studies have described high performance of MRI (1, 4, 6) or 3D TVUS (19) in assessing ovarian volume and antral follicle count, but with no direct comparisons between the modalities.

Ovarian volume and/or antral follicle count by TVUS is one of three diagnostic criteria for PCOS (12). The generally used reference standard in estimation of ovarian volume is the calculation of the formula *ovarian length x width x height x 0.5*, as measured by TVUS. Unexpectedly, in the present study the mean ovarian volumes assessed in this manner by 2D

MRI were significantly smaller as compared to assessment by 2D TVUS. One contributing factor for this discrepancy may be that MRI provided orthogonal planes that were fixed to the position of the body in the supine position on the examination table, in contrast to the real-time possibility of TVUS to adapt the orthogonal planes of the ovary independently of the body position. Ovarian volumes were also smaller assessed by 2D MRI as compared to 3D MRI. We do not know the true volumes of the ovaries, lacking surgical specimens as the gold standard, but it seems reasonable that estimation by the 3D MRI technique may be the most relevant one, since the ovaries do not always have the shape of an ellipsoid body, which is the basis for the formula used for volume assessment at TVUS. There may be irregularities in the surface of an ovary and it may sometimes have a curved shape, similar to the shape of a kidney (Fig. 7). With MRI, the manual outlining of the peripheral contour of the ovary when using the semi-automatic software to calculate the ovarian volume, make this technique unaffected by non-ellipsoid shape errors.

The mean follicle count in the smallest size interval of 1-3 mm was significantly higher when assessed by 2D MRI as compared with 3D TVUS, while it was lower in the intervals of 4-6 and 7-9 mm. The reason is probably an inability of the semi-automatic 3D TVUS technique to separate two or more adjacent small follicles, which causes it to count them as a single larger follicle in some cases. The manual counting in at least two different planes on 2D MRI images is probably superior in separating neighboring follicles, though partial volume effects between two slices is a difficulty in the interpretation. Follicular cysts 10 mm or larger were infrequent.

The interobserver agreement for MRI estimation of ovarian volume was very good and for total follicle count good. For follicle counts in the size interval 1-3 mm the interobserver agreement was moderate, and in the size interval 4-6 mm and 7-9 mm it was poor, probably indicating difficulties in determining if two or more follicles are situated close together or

regarded as a single larger follicle, and may also reflect differences in interpretation by an experienced radiologist (Observer 1) and a radiologist with limited previous experience in MRI reading (Observer 2). The data for medium sized and large follicles must be interpreted with care, as the numbers of follicles in these size ranges were small. The intraobserver analysis, on the other hand, showed very good agreement between most observations made one year apart, suggesting a reasonable robustness of the MRI method.

Our results suggest that MRI may be of value to shed further light on ovarian morphology and function, in e.g. PCOS and infertility (ovarian reserve). However, there are some limitations in the present study. First, there was no definite gold standard available, as this would require surgical removal and patho-anatomical analysis of the ovaries after imaging, a study design that is difficult to obtain. Second, antral follicle count by TVUS was limited to the 3D technique, as manual follicle count by 2D TVUS was not available. Third, there were no 3D TVUS ovarian volume estimations available to be compared to 3D MRI.

In clinical practice, TVUS remains the first line imaging modality to estimate ovarian volume and antral follicle count due to its simplicity and availability. However, the limitations in 3D identification of small follicles and differences in volume estimates as compared to MRI should be acknowledged. In research settings with high demands on methods for analyzing ovarian morphology in detail, the present study suggests that MRI should be the modality of first choice.

In conclusion, 2D MRI reveals more antral follicles, especially of small size, than semi-automatic estimates by 3D TVUS. Ovarian volume estimation by MRI provides smaller volumes than by the reference standard 2D TVUS. Ovarian volume assessed by 3D MRI, allowing independence of non-ellipsoid ovarian shape measurement errors, provides volumes

closer to 2D TVUS values than 2D MRI does. Measurements of ovarian volume and total follicle count by 2D MRI have an adequate intra- and interobserver agreement.

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## FIGURE LEGENDS

**Figure 1.** 3D TVUS of an ovary, illustrating the automated analysis of antral follicles using the Sono-Automatic Volume Count technique. The software individually color codes follicles in pre-defined size intervals and provides measurements of their diameters, volume and number.

**Figure 2 a and b.** T2-weighted transaxial (a) and coronal (b) MR images illustrating volume estimation of the left ovary by measuring the maximal diameter in three orthogonal directions. The ovarian volume is calculated as  $2.0 \times 2.1 \times 3.5 \times 0.523 = 7.7 \text{ cm}^3$ .

**Figure 3 a and b.** T2-weighted MR image in the transaxial plane with a polycystic right ovary outlined by a digital caliper (a) and “dissected” by use of the workstation’s semi-automatic software utilizing a seeding procedure for volume estimation (b).

**Figure 4.** Bland-Altman plot showing means and differences of ovarian volume measurements by transvaginal ultrasonography and 2D MRI. Solid line represents the mean of the differences, dashed lines represent the 95% coverage interval of the differences.

**Figure 5.** Bland-Altman plot showing means and differences of ovarian volume measurements by transvaginal ultrasonography and 3D MRI. Solid line represents the mean of the differences, dashed lines represent the 95% coverage interval of the differences.

**Figure 6.** Bland-Altman plot showing means and differences of ovarian volume measurements by 3D MRI and 2D MRI. Solid line represents the mean of the differences, dashed lines represent the 95% coverage interval of the differences.

**Figure 7.** T2-weighted MR image in the coronal plane in a women with PCOS, visualizing kidney-shaped polycystic ovaries.

## TABLES

**Table 1.** 2D TVUS, 2D MRI, and 3D MRI volume measurements in ovaries ( $n=172$ )

| Method  | Median ovarian volume (range) | Mean ovarian volume $\pm$ SD |
|---------|-------------------------------|------------------------------|
| 2D TVUS | 11.9 (3.7 - 49.2)             | 13.1 $\pm$ 6.4               |
| 2D MRI  | 9.1 (2.2 - 26.6)              | 9.6 $\pm$ 4.1                |
| 3D MRI  | 11.7 (3.2 - 25.7)             | 11.4 $\pm$ 4.5               |

Volume values are in ml. SD is standard deviation.  
2D ovarian volume is  $0.523 \times \text{length} \times \text{width} \times \text{height}$ .

**Table 2.** Comparison between 2D TVUS, 2D MRI, and 3D MRI volume measurements in ovaries ( $n=172$ )

| Method comparison | <i>P</i> -value  | Mean difference<br>± SD | Correlation coefficient |
|-------------------|------------------|-------------------------|-------------------------|
| 2D TVUS - 2D MRI  | <b>&lt;0.001</b> | 3.5 ± 4.6               | 0.702***                |
| 2D TVUS - 3D MRI  | <b>&lt;0.001</b> | 1.7 ± 4.4               | 0.749***                |
| 3D MRI - 2D MRI   | <b>&lt;0.001</b> | 1.9 ± 1.8               | 0.874***                |

Mean difference values are in ml. *P* values were determined by Wilcoxon signed ranks test. Correlation coefficients were assessed by Spearman's rho. \*\*\* $P < 0.001$ .

**Table 3.** 3D TVUS versus 2D MRI antral follicle counts in ovaries (*n*=169)

| Follicle count | 3D TVUS     |                     | 2D MRI      |                      | <i>P</i> - value | Mean difference in number of antral follicles ± SD | Correlation coefficient |
|----------------|-------------|---------------------|-------------|----------------------|------------------|--|-------------------------|
|                | Mean        | Median              | Mean        | Median               |                  |  |                         |
| 1 - 3 mm       | 7.8 ± 6.9   | 6 (0 - 49) [1312]   | 30.0 ± 19.0 | 25 (6 - 95) [5061]   | <b>&lt;0.001</b> | -22.2 ± 17.6                                       | 0.428***                |
| 4 - 6 mm       | 11.3 ± 8.3  | 10 (0 - 58) [1909]  | 7.1 ± 4.7   | 6 (0 - 29) [1202]    | <b>&lt;0.001</b> | 4.2 ± 6.9  | 0.480***                |
| 7 - 9 mm       | 3.6 ± 3.2   | 3 (0 - 20) [610]    | 0.5 ± 1.1   | 0 (0 - 9) [91]       | <b>&lt;0.001</b> | 3.1 ± 3.1  | 0.210**                 |
| 10 - 12 mm     | 0.6 ± 1.0   | 0 (0 - 4) [105]     | 0.1 ± 0.5   | 0 (0 - 3) [24]       | <b>&lt;0.001</b> | 0.5 ± 1.0  | 0.281***                |
| 13 - 15 mm     | 0.1 ± 0.4   | 0 (0 - 2) [22]      | 0.1 ± 0.2   | 0 (0 - 1) [8]        | <b>0.016</b>     | 0.1 ± 0.4  | -0.082                  |
| 16 - 18 mm     | 0.1 ± 0.3   | 0 (0 - 1) [14]      | 0.0 ± 0.2   | 0 (0 - 1) [5]        | <b>0.020</b>     | 0.1 ± 0.3  | 0.201**                 |
| 19 - 21 mm     | 0.0 ± 0.2   | 0 (0 - 1) [4]       | 0.0 ± 0.2   | 0 (0 - 1) [4]        | 1.000            | 0.0 ± 0.2  | 0.232**                 |
| ≥ 22 mm        | 0.1 ± 0.3   | 0 (0 - 1) [11]      | 0.1 ± 0.2   | 0 (0 - 1) [10]       | 0.564            | 0.0 ± 0.1  | 0.849***                |
| TFC            | 23.6 ± 14.9 | 21 (3 - 110) [3987] | 37.9 ± 20.3 | 33 (12 - 102) [6405] | <b>&lt;0.001</b> | -14.3 ± 16.2                                       | 0.658***                |

TFC is total follicle count. Method column values are median with range in parentheses and in total counted follicles within each size interval in square brackets. *P* values were determined by Wilcoxon signed ranks test. SD is standard deviation. Correlation coefficients were assessed by Spearman's rho. \*\**P* < 0.01, \*\*\**P* < 0.001.

**Table 4.** The intraobserver agreement of 2D MRI measurements of ovarian volume and follicle counts

| Variable       | ICC   | 95% CI        |
|----------------|-------|---------------|
| Ovarian volume | 0.995 | 0.990 - 0.998 |
| FC 1 - 3 mm    | 0.955 | 0.908 - 0.978 |
| FC 4 - 6 mm    | 0.859 | 0.726 - 0.930 |
| FC 7 - 9 mm    | 0.480 | 0.160 - 0.711 |
| TFC            | 0.874 | 0.755 - 0.938 |

FC is follicle count. TFC is total follicle count. ICC are single measures intraclass correlation coefficients. CI is confidence intervals.

**Table 5.** The interobserver agreement of 2D MRI measurements of ovarian volume and follicle counts

| Variable       | ICC   | 95% CI         |
|----------------|-------|----------------|
| Ovarian volume | 0.980 | 0.956 - 0.990  |
| FC 1 - 3 mm    | 0.687 | 0.047 - 0.885  |
| FC 4 - 6 mm    | 0.474 | 0.137 - 0.711  |
| FC 7 - 9 mm    | 0.119 | -0.208 - 0.438 |
| TFC            | 0.772 | 0.340 - 0.909  |

FC is follicle count. TFC is total follicle count. ICC are single measures intraclass correlation coefficients. CI is confidence intervals.