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REVIEW ARTICLE

Echographic Evidence of Follicle Development and Maturation

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ABSTRACT

Monitoring of individual follicles during the menstrual cycle demonstrates in a non-invasive way the changes in their number and position during the early and the late follicular phase and the luteal phase. The differences in relations between the follicles near the dominant follicle can be demonstrated with the same technique using 3D reconstruction of the ovary.

Recognition of the follicle growth pattern has a prognostic value for the outcome of assisted reproduction methods. Follicular diameter and changes in growth patterns are more important than follicular wall thickness as parameters having an impact on clinical success.

An increased perifollicular blood flow can be measured in the perifollicular period using color and pulsed Doppler. Automated estimation of blood volume around the ovarian follicles brought a new concept to this area. Results confirm the observation that vascularity around the follicle is intense in the periovulatory period.

From our results we can hypothesize that those follicles containing oocytes able to produce pregnancy have a prominent and more uniform perifollicular vascular network .

Keywords: Ultrasound, Ovarian monitoring, Follicle growth, Natural cycle, Controlled ovarian hyperstimulation, Perifollicular vascularization Monitoring.

INTRODUCTION

Kratochwil et al[1] were the first to describe and illustrate in 1972 that ovaries could be visualized by ultrasound and that follicles could also be identified. Following this initial report, the first systematic investigations with ultrasonographic demonstration of follicular development during spontaneous and stimulated cycles were performed in 1977.[2] Since then, several authors have confirmed the initial observations, and ultrasound provides a simple and non-invasive insight into the physiological changes during the menstrual cycle, also allowing accurate and reproducible investigations of follicular size, development and growth during the follicular phase.

Folliculogenesis is a constant process, which starts in the embryogenic period and ends with the disappearance of the last functional follicle in the period of menopause.

Sonographic Indices of Follicle Growth and Ovulation

For the purpose of reproducible and reliable data collection, it is common to take a follicular diameter of $\geq$2 mm as the lower visible limit. Secondary antral follicles, approximately 2 mm in diameter, are the first follicular structures that may be visualized and investigated reliably by the common US device.

The antral follicle count has clinical importance for estimating ovarian age and predicting its reactivity on controlled ovarian hyperstimulation.

Antral follicle *growth* in the early follicular phase has clinical importance in appropriate timing of selected follicles for aspiration of immature human oocytes for *in vitro* maturation (IVM), prediction of cycle outcome in a natural cycle or controlled ovarian hyperstimulation (COH) for IVF or intrauterine insemination (IUI).

Before the study of Gore et al[3] in the population of healthy volunteers, there had been no previous studies, which had attempted to survey the entire visible antral follicle population of the ovary noninvasively.

Gore et al[4] developed the method to identify, map, characterize and monitor growth, movement, positioning and interaction of individual follicles noninvasively using 2D ultrasound imaging along with computer modeling, which enabled 3D modeling of the ovary. They also introduced a set of visual criteria for the prediction of follicle status and cycle outcome (ovulatory *vs* nonovulatory) by investigating growth dynamics of the entire visible antral follicle population during natural, spontaneous cycles. In this study, ovulatory cycles were used as a reference for normality and these were characterized and compared with those cycles, which did not result in ovulation. They examined in detail the population of small follicles, restricting the analysis to the ovulatory cycles. An important finding was the drastic reduction of the antral follicle population at the end of the luteal phase, followed by a rapid increase in the population (new antral follicle growth). A new

group of growing follicles may signal intense competition for dominance. These findings suggest that the population of follicles from which a dominant follicle arises is at the start of the new cycle and is preantral or early antral (< 2 mm) sizes, not detectable with ultrasonography. The authors were able to identify the dominant follicle before it became the largest follicle, and demonstrate that the first largest follicle observed was not usually the ultimate dominant follicle.

Visible characteristics of antral follicles mapped in the ovary, used by these authors for the prediction of the ultimate fate of the follicle are: Size (the largest diameter of the follicle), shape (round, oval, rectangular, triangular), echogenicity (high, medium, low) and antral edge quality (smooth, intermediate, rough). *Subdominant follicles* (antral follicles that would not gain dominance) had the least regular shape and antral edge, the smallest size by definition and the highest echogenicity in comparison to all monitored dominant follicles throughout the follicular phase. *Ovulatory follicles* (antral follicles that would ultimately ovulate) could be distinguished from atretic follicles by their round shape and from luteinized unruptured follicles by their middle range echogenicity. By the end of the follicular phase, their antral edge became smoother. *Atretic dominant follicles* had the least regular shape, but they were the largest follicles in the early follicular phase. Their antral edge then became more irregular, while their echogenicity remained at the same level. *Luteinized unruptured follicles* (dominant follicles that would not ovulate, but would become luteinized) could be distinguished from other dominant follicles throughout the follicular phase by their regular shape, smooth antral edge and very low echogenicity.

The selection of the dominant ovulatory follicle occurs before day 5 of the cycle, after day 5 to 7 its dominance becomes apparent by its continuously increased size by 2 mm per day.

By distinguishing antral follicles from those identified later on as dominant and those where atresia will occur in the next few days of development (challenger follicles), ultrasound offers the possibility of new information about the behavior of such cohorts of antral follicles (> 6 mm in diameter).[3,4] The results of this study showed that antral follicle development was not restricted to a particular location in the ovary. The apparent randomization of location may provide a developmental advantage for the follicles. Using the comparison of the day-by-day ultrasonographic scan with the 3D model of the same ovary, it was established that the dominant follicles subsequently appeared to reduce the number of neighboring challenger follicles. This result suggests that paracrine secretion of the dominant follicle was the source for the localized effect providing increased nutrient level and space at the critical growth stage for dominant follicles. The dominant follicle has a linear growth rate between 1.4 and 2.2 mm per day until the day of the LH peak, at the time of the LH surge it measures between 18.1 and 22.6 mm. After the peak, growth increases very quickly.[5]

Sequential ultrasonographic follow-up of follicle growth and recognition of different growth patterns was published by Nayudu et al.[6] The first US scan was done between day 8 and 10 of the cycle to determine whether a pattern of growth is common in the cohort of retrieved follicles related to conception. They observed 107 stimulated cycles and constructed individual profiles for each follicle. Parameters of follicle growth pattern have been suggested to be associated with or predictive of follicle quality.

The growth rate of 2 mm per day quoted generally is certainly too low. In our previous study, we analyzed the growth rate in 101 IUI cycles. In pregnant patients a rapid daily follicular growth rate (> 2.3 mm/day) was present more frequently than in nonconceivers.[7]

Sonographic Indices of Ovulation

The preovulatory follicle undergoes great changes during the last seven days preceding ovulation: An increase in size and granulosa cell thickness and increased perifollicular vascularization in the theca layer. Changes in follicular diameter and volume are an ultrasonographic manifestation of changes in the granulosa cell compartment initiated by LH. This echographic finding reflects the echographic manifestation of physiologic reactivity of granulosa cells (intensive division) and the increase of follicular fluid, which correspond only to an increase of serum transudate through the granulosa. The thickness of the granulosa wall is in direct relation with health or atresia of the follicle, not only during the follicular phase but also shortly before ovulation. A thin follicular wall is suggestive of an atretic follicle.

Several ultrasonographic parameters, which are indicative of ovulation, have been reviewed for sensitivity and specificity in the study of Ecochard et al.[8] If we accept the definition of the day of ovulation as the day of maximal follicular growth or as the day of follicle rupture, the key sonographic indices of ovulation are: Disappearance of or sudden decrease in follicle size (the most frequent sign of ovulation with the sensitivity of 84%), appearance of ultrasonic echoes in the follicle (the sign which is not quite reliable, because its value rises both before and after ovulation—the proportion of cycles with echoes in the follicle increases gradually during the three days preceding ovulation, but there is also a sharp increase of this proportion on the first postovulatory day), irregularity of follicle walls (sensitivity of this sign, which is present in almost 70% of cycles, is much higher than that of the appearance of intrafollicular echoes), free fluid in the pouch of Douglas [despite the fact that free fluid could be seen in the cul-de-sac during all phases of the cycle, it is a rare finding (3-11% of the cycles) during the preovulatory phase, but much more common (77%) on the day of ovulation].

Angiographic Studies of Preovulatory Follicles

Perifollicular blood flow velocities gradually increase in the periovulatory period. This increase starts approximately 29 hours before ovulation and continues for at least 72 hours

later, and that may be the consequence of the penetration of blood vessels into the granulosa cell layer. In the same period, the pulsatility index (PI) remains relatively constant, and the vascular resistance index in perifollicular vessels shows low to moderate values. These findings suggest a marked increase in blood flow at the periovulatory period (Fig. 1).[9,10]

The study of Jarvela et al[11] was the first one to utilize both 3D sonography and power Doppler for the evaluation of vascularization and blood flow during the late follicular phase in women with normal ovulation. No differences were detected in the values of vascularization indices between the dominant and the nondominant ovaries, suggesting that the mean blood flow in both ovaries was equal.

An increased perifollicular blood flow can be measured in the perifollicular period using color and pulsed Doppler. Automated estimation of blood volume around the ovarian follicles brought a new concept to this area. Our results confirm the observation that vascularity around the follicle is intense in the periovulatory period. The blood volume does not differ between follicles containing an oocyte and those with no oocyte in the aspirate, or a nonfertilizable oocyte. From our results we can hypothesize that those follicles containing oocytes able to produce pregnancy have a more uniform perifollicular vascular network.[12]

Early angiographic studies carried out by color Doppler ultrasonography in natural cycles showed that the main characteristics of blood flow in perifollicular tissue of nondominant growing antral follicles were low velocity and high resistance (Fig. 2).[13]

Quantitative Estimation of Blood and Vessels around the Follicle

Studies of perifollicular blood flow in small groups of natural cycles demonstrated an increase in peak systolic velocity (PSV) and a tendency of the pulsatility index (PI) to decrease.[9,14,15] In other studies, the authors report a statistically significant increase in PSV in perifollicular vessels, but no change in PI.[9,14] In the later study of Gavric Lovrec et al[16] carried out in a larger group (a total of 178 cycles) of unstimulated cycles, no statistically significant increase in PSV between fertilization and nonfertilization cycles was demonstrated, but rather a decrease in PI

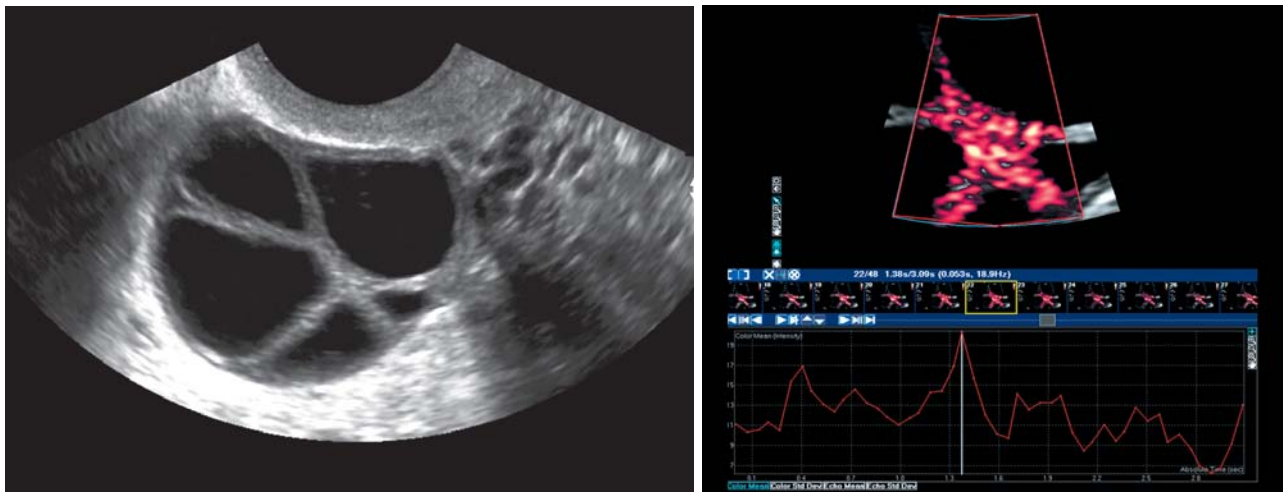

Fig. 1: Multifollicular development and vascularization of intrafollicular septa

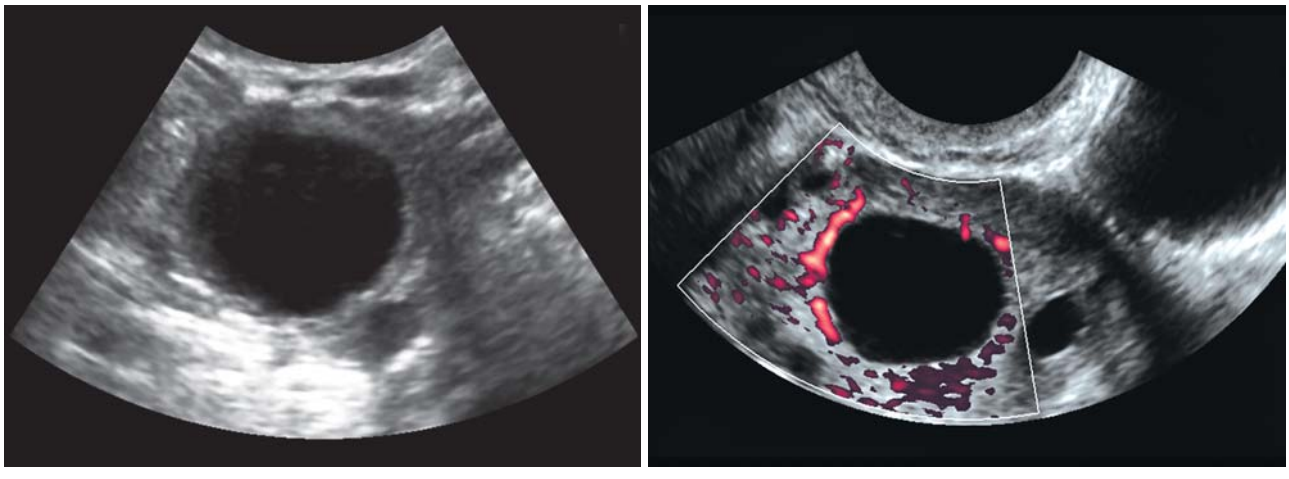

Fig. 2: Single follicle from natural cycle and perifollicular vascularization on 2D image

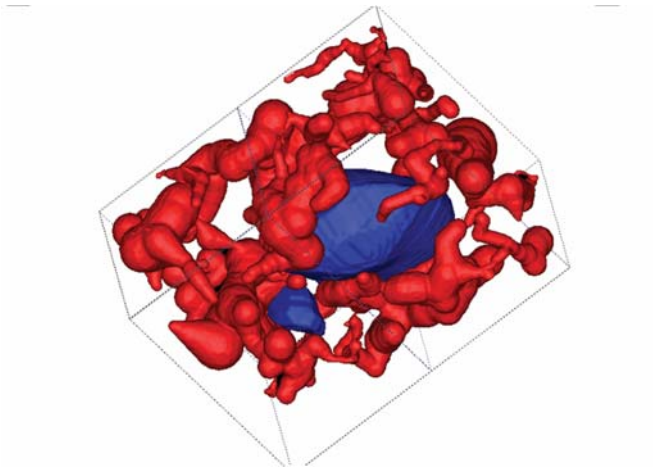

Fig. 3: 3D reconstruction of perifollicular vascularization in single follicle from natural cycle (*Courtesy by FERI, University Maribor*)

and resistance index (RI) was shown. The study investigated the usefulness of the percentage of blood volume showing a flow signal (VFS) inside a 5 mm capsule of perifollicular tissue of the dominant preovulatory follicle as a predictor of the outcome of unstimulated IVF/ICSI cycles.[12] The more uniform vascular network in the capsule was a more frequent finding than average in cycles with implantation compared to nonimplantation cycles, but this difference only reached borderline statistical significance.

A 3D reconstruction of vessels is necessary in order to track them and to divide the total blood volume into the individual contributions of each vessel forming part of this vascular network. The tracking of blood vessels is based on the comparison of overlapping regions containing blood in the neighboring images of an ultrasound scan. The computer program automatically generates several graphical and numerical results. A coarse display depicts the 3D reconstruction of the dominant follicle and perifollicular capillary network. The information is given on the most important blood vessels, the volumetric portion of blood flow through them, and their spatial position with respect to the follicle. The volumetric ratio among blood vessels, the follicle and the surrounding tissue in the 5 mm capsule are also displayed. The volume of blood in individual vessels of the perifollicular network was expressed as blood volume supplied from each individual vessel inside the follicle capsule to the total perifollicular blood volume.

Several studies have tried to find a correlation between the degree of vascularization of the follicle and oocyte quality. In future, in order to improve the pregnancy rate per oocyte pick-up in a natural cycle, 3D power Doppler should be considered as a method helping to decide which cycle should be cancelled from oocyte pick-up. Furthermore, the non-invasive character of 3D power Doppler technique makes this examination more suitable for evaluation of natural cycles.

Clinical implications of the described US technique in assessment of oocyte quality may change everyday practice in medically assisted reproduction (Fig. 3).

After the administration of hCG, the dominance of single feeding vessels decreases, resulting in more balanced blood inflow through several feeding vessels with a similar percentage of total volume of vessels showing flow signal.[17]

Sonography in the Prediction of Success of IVF Cycles

To improve the pregnancy rate with IVF cycles, it is important to identify the factors that are able to predict the outcome of the monitored cycle and the success of the IVF procedure. In a series of studies, a great number of nonsonographic variables with possible predictive values have been investigated and classified into three large categories: Maternal age, ovarian reserve and past reproductive history.

Recognition of the follicle growth pattern has a prognostic value for the outcome of assisted reproduction methods. Follicular diameter and changes in growth patterns are more important than follicular wall thickness as parameters having an impact on clinical success.

Growth patterns in nonstimulated cycles are important, but only in relation with estradiol monitoring for the appropriate time of hCG administration. Unstimulated cycles monitored with ultrasound and the combination of serum E2 and urinary LH can produce an acceptable pregnancy rate after IVF and ICSI. hCG should be administered when serum E2 is > 0.49 nmol/L and follicle diameter at least 15 mm. A higher pregnancy rate and lower cancellation rate can be obtained when hCG is applied in lower values of serum E2 and smaller follicle diameters, below the diameter believed to be of preovulatory size.[19]

Different growth patterns during the follicular phase of the cycle have different values in different types of stimulation protocols. The critical points for administration of hCG should differ in GnRH agonist and GnRH antagonist protocols. Some authors advocate different decisions for hCG administration in stimulation protocols with FSH and also in HMG. Recording of follicle growth and the day when follicle selection started is important for GnRH antagonist administration, which is the main difference between so-called "fix" and "flexible" antagonist protocols.[18] In flexible protocols, great importance is attributed to follicle growth dynamics. The antagonist should be administered at the moment when the follicles are selected from the cohort and they reach 12 to 14 mm in diameter.

Ultrasonographic parameters as predictors of IVF success have also been investigated. Introduction of color Doppler, power Doppler and 3D ultrasound devices have allowed research of a very wide spectrum of potential predictors. Sonographic parameters with predictive value on cycle outcome have been tested mostly for stimulated cycles, and only few studies are focused exclusively on natural cycles.

Ultrasound Monitoring in Natural Cycles

The term natural cycle is used to describe spontaneous, unstimulated cycles from which oocytes are recovered for IVF after human chorionic gonadotropin (hCG) administration to replace and precede the natural luteinizing hormone (LH)

surge.[20] IVF in natural cycles is an accepted method for treatment of infertility in selected patients,[21,22] and their monitoring and clinical application has greatly extended our knowledge of human reproductive physiology. Despite the fact that those cycles have several advantages over stimulated ones, high failure rate at each step in the process and the unacceptably low delivery rate per oocyte recovery attempt compared to stimulated cycles are the main reasons why they are not widely used in IVF programs.[23]

In IVF programs where single follicle development in natural cycles was monitored by ultrasound only, the main problem was an unwanted spontaneous LH surge. Even when folliculometry was repeated every second day until the follicle reached a diameter of 16 mm and then carried out daily, when the average diameter of the dominant follicle reached 18 mm, in 40% of cycles a spontaneous LH surge or ovulation was observed before oocyte pick-up.[22]

Based on the results of our previous study,[19] we modified the criteria for hCG application to those when the follicle reaches at least 15 mm in mean diameter and serum E2 is 0.49 nmol/L. Despite such low values of serum E2 and the small mean follicle diameter, the aspirated oocytes were suitable for IVF/ICSI with similar fertilization rate and implantation rate of cleavage embryos with spontaneous LH surge or ovulation before OPU in less than 10% of cases.

Ultrasound as the only Monitoring Tool for Assisted Reproduction

Before ultrasonography became a routine method for monitoring of follicle development, this role was attributed to serum estradiol (E2). After it was demonstrated that a direct correlation exists between follicular growth and E2 level, ultrasound was introduced for follicular growth, which previously had been so indirectly evident. Ultrasound correlated precisely with E2 assays and with the number of mature follicles.

The growing follicle matures and secretes increasing levels of estradiol (E2). E2 affects the target organs and promotes proliferation of the endometrium, increasing its thickness. Clear associations between the diameter of the dominant follicle and the levels of E2, and between the levels of E2 and endometrial thickness inspired some authors to try using ultrasound as the only monitoring tool for IVF cycles. Another important reason that made this approach attractive was the economic benefit for the patient. In 1985 Nilson et al[24] suggested that ultrasound alone is sufficient to estimate follicular maturity and hence the administration of hCG which determines ovulation. Vlaisavljevic et al[25,26] advocate a simplification of ultrasound monitoring where COH is adjusted to the woman's needs.

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