Article

Improved monitoring of ovarian stimulation using 3D transvaginal ultrasound plus automated volume count

Dr Maximilian Murtinger received his MD at the University of Vienna in 1986. After specialization as a trauma surgeon in 2003, he changed to the field of reproductive medicine and endocrinology and is currently finishing his specialization in obstetrics and gynecology. His current interest is in ultrasound techniques, especially related to follicular measurements in stimulated cycles and in establishing clinical standards related to the quality criteria set by the European Union directive on tissues and cells (2004/23/EC). In addition, Dr Murtinger is developing requirements for software used in obstetrics and gynecology, especially for reproductive medicine and endocrinology.

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Abstract

Two-dimensional transvaginal ultrasound (2D) is typically performed to monitor follicle growth in IVF and to determine the optimal time for administering human chorionic gonadotrophin. However, 2D only provides an approximation of the real volume of follicles and therefore cannot be used to guarantee standards for follicular measurement. The automated measurement of follicular size in three dimensions (3D) using a software programme that identifies and quantifies hypoechoic regions within a 3D dataset might provide an objective, fast, valid and reliable standard for such measurements. A prospective controlled study (group I: 20 patients, 2D; group II: 20 patients, 3D) investigated how the criteria for triggering oocyte maturation that are normally used in 2D compare to the new and more accurate method of measuring follicles using 3D-based automated volume count. Significantly more oocytes were fertilized (group 1: 7.1 ± 4.5, group 2: 11.5 ± 6.4; $P < 0.03$) when using 3D technology and automated volume count. The study assumes that the automated volume count more closely mirrors the biological reality, which means that it can also be used to guarantee the quality standards established by the European Union directive on tissues and cells (2004/23/EC). This new technology therefore holds great promise of becoming the new standard for monitoring follicular growth in IVF.

Keywords: *automated volume count, in-vitro fertilization, monitoring, ovarian stimulation, three-dimensional ultrasound*

Introduction

Two-dimensional transvaginal ultrasound (2D) is typically performed for monitoring multifollicular recruitment and growth in ovarian stimulation as part of IVF treatment. In order to calculate the optimal time for administering human chorionic gonadotrophin (HCG) to trigger follicle and oocyte maturation before oocyte retrieval, 2D measurements of follicles are usually made and their mean is taken as the true follicle diameter. However, 2D only provides an approximation of the real volume of follicles and therefore cannot be used to guarantee standards for follicle tracking, that is, follicular measurement (Shmorgun *et al.*, 2009). While some observers rely on a single 'best' estimate, others use two or three measurements in one or more planes. As the number of follicles increases, it becomes increasingly difficult to ensure that each and every follicle is included in the measurement. This diminishes the validity and reliability of 2D follicular diameter measurements (Forman *et al.*, 1991). Furthermore, the time required to take these measurements increases with the number of follicles present.

The automated measurement of follicular size might provide an objective, fast, valid and reliable standard for such

measurements. An automated volume count software programme has become commercially available. This programme identifies and quantifies hypoechoic regions within a 3D dataset (**Figure 1**) and provides an automatic estimation of their absolute volumes, calculating back from each volume to a mean diameter of a perfect sphere (Raine-Fenning *et al.*, 2008a).

It has been shown that the follicular volumes measured and calculated with the automated volume count (AVC) are in accordance with the aspirated volumes (Raine-Fenning *et al.*, 2007) and that they also provide more accurate data than traditional 2D measurements (Raine-Fenning *et al.*, 2008b).

The aim of this study was to compare the traditional 2D method with the new 3D-based AVC with regard to the number of oocytes retrieved, the fertilization rate, the number of embryos developing to the blastocyst stage on day 5 and the pregnancy rate. Therefore, this study determined how the criteria for triggering oocyte maturation that are normally used in 2D compare to the new and more accurate method of measuring follicles using 3D-based AVC.

Materials and methods

This prospective controlled study, conducted between February and December 2008, enrolled 40 patients who entered the study center's IVF programme. Inclusion criteria were: women between 25 and 35 years of age who entered the IVF programme because of male factor infertility [excluding severe forms of oligoasthenoteratozoospermia (OAT), i.e. <1 million spermatozoa/ml, <10% motile spermatozoa and >90% pathological forms] with or without tubal factor. All women had to have a normal body mass index (<25 kg/m^2), a menstrual cycle length between 26 and 32 days, normal FSH and LH concentrations on day 3 and day 5 after menstruation in the last 6 months and no prior history of missed abortion or failed IVF/ICSI. The patients were assigned to one of two groups (group I: decision for time of HCG administration based on 2D ultrasound; group II: decision based on 3D AVC) in an alternating manner (assignment changed after each 10 patients). The criteria for triggering final oocyte maturation with HCG were the same in both groups and performed by the same physician. The aim was to have a maximum number of follicles between 16 and 22 mm at the time of HCG administration (Rosen *et al.*, 2008; Shmorgun *et al.*, 2009). Consequently, an ultrasound examination was performed every second day starting on day 6. The day of HCG administration was determined assuming a 1.5–2 mm follicular growth per day (Rossavik and Gibbons, 1986). Informed consent was obtained before the enrolment of each patient in the study.

Ovarian stimulation protocols and ultrasound monitoring

In order to achieve down-regulation of the pituitary gland, the gonadotrophin-releasing hormone (GnRH) long protocol was applied with 0.1 mg triptorelin s.c. daily

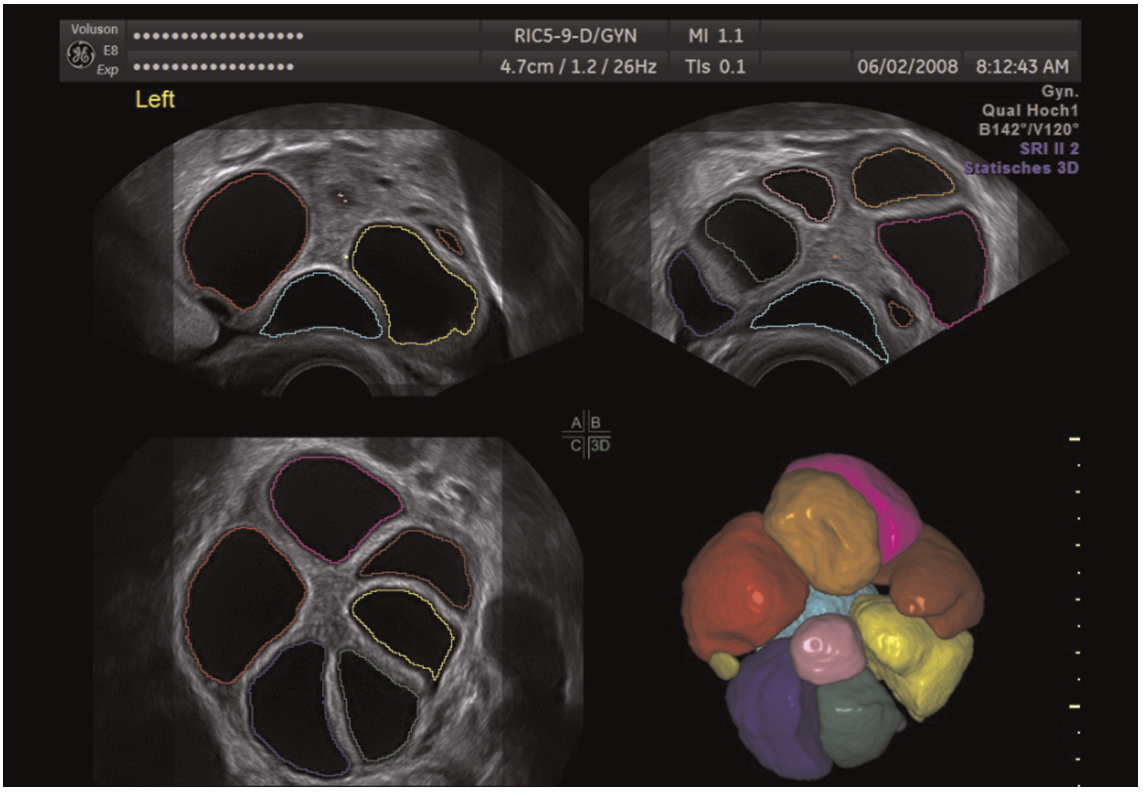

Figure 1. Representation of a stimulated ovary (bottom right) generated via 3D transvaginal ultrasound scanning plus automated volume count software 1 day before triggering final oocyte maturation.

(Decapeptyl; IPSEN Biotech, Paris, France), starting in the midluteal phase of the preceding cycle and continuing until the day of HCG injection. At the earliest opportunity, 12 days after onset of menstrual bleeding, gonadotrophin treatment was started using 150 IU highly purified human menopausal gonadotrophin (Menopur; Ferring Pharmaceuticals A/S, Ørestad City, Denmark) and 75 IU rFSH (Gonal-F; Serono Pharmaceuticals Ltd, Feltham, UK).

A transvaginal scan using a 3D/4D 5–9 MHz endocavity (transvaginal) probe was performed on all patients every second day, starting on day 6 of stimulation, by one investigator (VE) using a Voluson E8 (GE Medical Systems, Kretztechnik, Zipf, Austria). The scan was performed until the date for administering HCG had been determined (according to the criteria below) and assuming a 1.5–2 mm follicle growth per day (Rossavik and Gibbons, 1986).

A predefined probe programme with optimized settings guaranteed consistent and identical ultrasound parameters. The scan was performed in a modified Lloyd-Davies position, and patients had to have an empty bladder. Initial 2D with follicle measurements in two planes was followed by the application of a region of interest over the ovary, which defined the volume to be acquired in 3D. A motorized sweep of this region through 120° was then undertaken using the slow sweep mode. The resultant multiplanar display was examined to ensure that the entire ovary had been captured. A single acquisition was obtained for each ovary because this has been shown to provide reliable data (Raine-Fenning *et al.*, 2004). Data were saved to the hard drive of the ultrasound machine and subsequently transferred to a personal computer without any data compression via a DICOM network. The volume of the follicles was automatically calculated using SonoAVC™ software (GE Medical Systems, Kretztechnik, Austria).

In group I, 10,000 IU HCG (Profasi; Serono Pharmaceuticals Ltd, Feltham, UK) were administered when a cohort of four or more follicles reached a diameter between 16 and 22 mm measured by 2D ultrasound and assuming a 1.5–2 mm growth per day.

In group II, HCG application depended on the AVC of the follicles (true volume). By applying the sphere formula ($d = 2\ [3\sqrt{3}/4V/]$, where d is the diameter and V is the volume), AVC was used to calculate the true diameter of the follicles. The same criteria for triggering final oocyte maturation in group I were applied to these calculated true diameters. Oocytes were collected 34–35 h after the administration of 10,000 IU of HCG and classified according to Veeck (1985). Luteal phase support consisted of administering 50 mg progesterone (Streuli, Richterswil, Switzerland) i.m. daily, starting from the day of oocyte retrieval until 16 days thereafter. This was continued until pregnancy occurred, which was defined as the presence of gestational sac(s) with fetal heart activities at 30 days after transfer.

In-vitro culture of embryos

Oocytes were incubated in IVF 20 medium (Scandinavian Science, Gothenborg, Sweden) and fertilized oocytes were cultured in groups in four-well multidishes (Nunc), each well containing 800 µl of non-sequential Global medium (LifeGlobal, Ontario, Canada) supplemented with 7.5% human serum albumin (LifeGlobal) at 37°C in a humidified atmosphere of 6% CO_2 in air. After routine ICSI (Vanderzwalmen *et al.*, 1996), all oocytes were checked 16–20 h later for the presence of two pronuclei. On day 3 of culture, the quality of the embryos was evaluated, followed by further culturing to the blastocyst stage.

On day 5, embryo quality was recorded and assessed according to the degree of blastocoele expansion and the quality of both the inner cell mass (ICM) and the trophectoderm. Embryo transfers were performed according to the clinic's standard practice. No more than two embryos were transferred.

Statistical analysis

Mann-Whitney *U*-test and the chi-squared test were applied to compare the different variables. Calculations were performed with the Statistical Package for Social Sciences 11.0 (SPSS Inc., USA). Statistical significance was set at $P < 0.05$.

Results

The study population comprised of 40 patients. As shown in **Table 1**, the two groups studied did not significantly differ in terms of maternal age (group I: 31.4 ± 3.5 versus group II: 31.7 ± 4.0), the number of oocytes retrieved (group I: 12.9 ± 5.8, group II: 16.1 ± 7.3), mature oocytes (group I: 10.1 ± 5.4, group II: 13.6 ± 7.0), immature oocytes (group I: 1.7 ± 1.9, group II: 1.6 ± 1.8) or mature oocytes per oocytes recovered (group I: 1.4 ± 0.5, group II: 1.2 ± 0.2), embryo development to day 5 (group I: 4.0 ± 2.0, group II: 5.3 ± 3.9), the mean number of embryos transferred (group I: 1.7 ± 0.1, group II: 1.6 ± 0.1) and the pregnancy rate (group I: 50%, group II: 45%). In group II, significantly more oocytes were fertilized (group I: 7.1 ± 4.5, group II: 11.5 ± 6.4; $P = 0.031$).

Discussion

As shown in this study, it is feasible to use 3D transvaginal ultrasound plus automated volume count for determining the day of HCG administration in IVF treatment based on the same criteria used in 2D ultrasound. Moreover, it seems that with this technique more occytes are retrieved and subsequently significantly more are fertilized on day 1. Besides that, 3D AVC took less time for follicular diameter measurement (data not shown) than the 2D measurement method, with no inter-observer variability. This fact might in future benefit the clinical workflow and increase patient throughput. Furthermore, the DICOM standard allows for data to be easily exported to a patient database.

The shorter examination time and objective follicle diameter measurement associated with 3D AVC, combined with reduced exposure to ultrasound due to the acquisition of

Table 1. Comparison of characteristics in the two study groups. Group I ($n = 20$) were monitored using 2-dimensional transvaginal ultrasound; group II ($n = 20$) were monitored using 3-dimensional transvaginal ultrasound with automated volume count.

Parameter	*Group I*	*Group II*
Age of women (years)	31.4 ± 3.5	31.7 ± 4.0
Oocytes recovered	12.9 ± 5.8	16.1 ± 7.3
Mature oocytes	10.1 ± 5.4	13.6 ± 7.0
Immature oocytes	1.7 ± 1.9	1.6 ± 1.8
Mature oocytes/oocytes recovered	1.4 ± 0.5	1.2 ± 0.2
Oocytes fertilized	7.1 ± 4.5[a]	11.5 ± 6.4[b]
Day-5 blastocysts	3.3 ± 2.0	4.6 ± 3.9
Embryos transferred	1.7 ± 0.1	1.6 ± 0.1
Pregnancy rate% (*n*)	50 (10)	45 (9)

Values are mean ± SD unless otherwise stated.
[a,b]$P = 0.031$; there were no other statistically significant differences between the study groups.

3D data (Jayaprakasan *et al.*, 2008), may confer clinical benefits to patients and guarantee the quality standards established by the European Union directive on tissues and cells (2004/23/EC). Additionally, 3D AVC is an objective and reproducible measurement method.

A general weakness of such studies is that the optimal day for triggering final oocyte maturation relies on rather weak criteria. These criteria are the results of studies (Wittmaack *et al.*, 1994; Ectors *et al.*, 1997) which show that the number of follicles between certain volumes correlates with the number of oocytes retrieved as well as their subsequent competence for development after fertilization. The strength of this study is that inter-observer variability was not given, which reduced the bias resulting from such weak criteria.

The average follicle diameter on the last ultrasound using 2D measurements was 15.1 compared with 13.1 when applying 3D AVC. However, these data carry no useful information, because the 3D AVC measures more small follicles. Therefore the question arises where to set the limits to calculate the average. Moreover, even if a cut-off were to be defined, no meaningful data would be gained because, in the study centre's experience, 2D and 3D AVC data do not compare well even when performed on the same patient and on the same day. It needs to be considered whether new criteria need to be defined that better suit the more precise measurements of 3D AVC. This question is subject of ongoing studies.

As shown recently (Shmorgun *et al.*, 2009), oocyte yield is best when the cohort of follicles lies between 16 and 22 mm; fewer oocytes are retrieved if the cohort of follicles is below or above this range. Additionally, this group showed that 3D data relate more closely to the number of oocytes retrieved compared with 2D data.

For this reason, it is assumed that 3D AVC more closely mirrors the biological reality (i.e. the cohort of mature oocytes which are believed to be in the group of follicles between certain volumes). This might explain why in group II more oocytes were found and significantly more oocytes fertilized on day 1. It remains speculative why these facts did not result in more embryos developing to day 5 or in higher pregnancy rates in group II. However, this is probably due to the stringent inclusion criteria (overall high rates of blastocyst development and pregnancy rates in this patient collective) and the fact that the sample sizes were too small to show statistical significance in these two parameters.

The 2D group had four patients who had blastocysts cryopreserved and the 3D group had three patients who had embryos cryopreserved (two patients at the blastocyst-stage and one patient with cryopreservation on day 1 due to ovarian hyperstimulation syndrome. The policy is to freeze excess embryos only if they fulfil certain quality criteria on day 5 or if ovarian hyperstimulation syndrome is present. Even when cryopreserving, the protocol aims for a fresh cycle. Thus, it is difficult to draw conclusions from cryocycles for this study. This is also the reason why these results were not included and hope that future studies will solve this issue, i.e. if the higher fertilization rate also translates into more blastocysts with a better cumulative pregnancy rate using 3D AVC.

It would be of special interest to further research whether the data gathered using 3D AVC may lead to better criteria for determining the optimal time of HCG administration. First steps in this direction may include an investigation into follicle growth. This will lead to better prognoses on the optimal time for HCG administration.

References

Ectors FJ, Vanderzwalmen P, Van Hoeck J *et al.* 1997 Relationship of human follicular diameter with oocyte fertilization and development after in-vitro fertilization or intracytoplasmic sperm injection. *Human Reproduction* **12**, 2002–2005.

Forman RG, Robinson J, Yudkin P *et al.* 1991 What is the true follicular diameter: an assessment of the reproducibility of transvaginal ultrasound monitoring in stimulated cycles. *Fertility and Sterility* **56**, 989–992.

Jayaprakasan K, Campbell BK, Clewes JS *et al.* 2008 Three-dimensional ultrasound improves the interobserver reliability of

antral follicle counts and facilitates increased clinical work flow. *Ultrasound in Obstetrics and Gynecology* **31**, 439–444.
Raine-Fenning N, Jayaprakasan K, Clewes J *et al.* 2008a SonoAVC: a novel method of automatic volume calculation. *Ultrasound in Obstetrics and Gynecology* **31**, 691–696.
Raine-Fenning N, Jayaprakasan K, Chamberlain S *et al.* 2008b Automated measurements of follicle diameter: a chance to standardize? *Fertility and Sterility* **91**(suppl. 4), 1469–1672.
Raine-Fenning N, Jayaprakasan K, Clewes J *et al.* 2007 Establishing the validity of a new technique that facilitates automated follicular volume measurement. *Ultrasound in Obstetrics and Gynecology* **30**, 393.
Raine-Fenning NJ, Campbell BK, Clewes JS *et al.* 2004 The interobserver reliability of three-dimensional power Doppler data acquisition within the female pelvis. *Ultrasound in Obstetrics and Gynecology* **23**, 501–508.
Rosen MP, Shen S, Dobson AT *et al.* 2008 A quantitative assessment of follicle size on oocyte developmental competence. *Fertility and Sterility* **90**, 684–690.
Rossavik IK, Gibbons WE 1986 Growth curve analyses of follicular growth in the in-vitro fertilization program. *Fertility and Sterility* **45**, 834–838.
Shmorgun D, Hughes E, Mohide P *et al.* 2009 Prospective cohort study of three- versus two-dimensional ultrasound for prediction of oocyte maturity. *Fertility and Sterility*, Epub ahead of print.
Vanderzwalmen P, Bertin G, Lejeune B *et al.* 1996 Two essential steps for a successful intracytoplasmic sperm injection: injection of immobilized spermatozoa after rupture of the oolema. *Human Reproduction* **11**, 540–547.
Veeck LL 1985 Extracorporeal maturation: Norfolk, 1984 *Annual of the New York Academy of Sciences* **442**, 357–367.
Wittmaack FM, Kreger DO, Blasco L *et al.* 1994 Effect of follicular size on oocyte retrieval, fertilization, cleavage, and embryo quality in in-vitro fertilization cycles: a 6-year data collection. *Fertility and Sterility* **62**, 1205–1210.

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