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.

Maximilian Murtinger1,3, Alexander Aburumieh1,3, Paul Rubner1, Verena Eichel1, Mathias H Zech1, Nicolas H Zech1,2,4
1IVF Centers Prof. Zech, Bregenz, Austria; 2Departments for Obstetrics and Gynecology, Unit of Gynecological Endocrinology and Reproductive Medicine, University of Graz, Austria; 3MM and AA contributed equally to this paper
4Correspondence: e-mail: n.zech@ivf.at

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/m²), 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 the 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 follicle growth per day.

In group II, HCG application depended on the AVC of the follicles (true volume). By applying the sphere formula \( V = \frac{4}{3} \pi d^3 \), 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 the degree of blastocoele expansion and the quality of 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 ± 5.2), the number of oocytes retrieved (group I: 12.9 ± 5.8, group II: 16.1 ± 7.6), mature oocytes (group I: 10.1 ± 5.2, 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 oocytes are retrieved and subsequently significantly more are fertilized on day 1. Besides that, 3D AVC took less time for follicle 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...
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., 2008) for determining the optimal time of HCG administration. This will lead to better prognoses on 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


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. 
Raine-Fenning N, Jayaprakasan K, Clewes J *et al*. 2008a
Raine-Fenning N, Jayaprakasan K, Chamberlain S *et al*. 2008b
Automated measurements of follicle diameter: a chance to 
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 
terobserver reliability of three-dimensional power Doppler 
data acquisition within the female pelvis. *Ultrasound in 
Rosen MP, Shen S, Dobson AT *et al*. 2008 A quantitative 
assessment of follicle size on oocyte developmental competence. 
Rossavik IK, Gibbons WE 1986 Growth curve analyses of 
fOLLricular growth in the in-vitro fertilization program. *Fertility 
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.
steps for a successful intracytoplasmic sperm injection: injection 
of immobilized spermatozoa after rupture of the oolema. 
Veeck LL 1985 Extracorporeal maturation: Norfolk, 1984 *Annual 
Wittmaack FM, Kreger DO, Blasco L *et al*. 1994 Effect of follicular 
size on oocyte retrieval, fertilization, cleavage, and embryo 

Declaration: The authors report no financial or commercial 
conflicts of interest.

Received 5 February 2009; refereed 19 February 2009; accepted 22 
July 2009.