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● *Original Contribution*

A NOVEL TECHNIQUE FOR THE SEMI-AUTOMATED MEASUREMENT OF EMBRYO VOLUME: AN INTRAOBSERVER RELIABILITY STUDY

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Abstract—The aim was to assess intraobserver reliability of a new semi-automated technique of embryo volumetry. Power calculations suggested 46 subjects with viable, singleton pregnancies were required for reliability analysis. Crown rump length (CRL) of each embryo was analyzed using 2-D and a 3-D dataset acquired using transvaginal ultrasound. Virtual organ computer-aided analysis (VOCAL) was used to calculate volume of gestation sac (GSV) and yolk sac (YSV) and SonoAVC (sonography-based automated volume count) was used to quantify fluid volume (FV). Embryo volume was calculated by subtracting FV and YSV from GSV. Each dataset was measured twice. Reliability was assessed using Bland-Altman plots and intraclass correlation coefficients (ICCs). Fifty-two datasets were analyzed. Median embryo volume was 1.8cm^3 (0.1 to 8.1 cm^3); median gestational age 7 + 4 weeks; median CRL 13 mm (2 to 29 mm). Mean difference of embryo volume measurements was 0.1cm^3 (limits of agreement [LOA] –0.3 to 0.4 cm^3); multiples of mean (MoM) 0.38; mean difference of CRL measurements 0.3 mm (LOA –1.4 to 2.0 mm), MoM = 0.26. ICC for embryo volume was 0.999 (95% CI 0.998 to 0.999), confirming excellent intraobserver agreement. ICC for CRL was 0.996 (95% CI 0.991 to 0.998). Regression analysis showed good correlation between embryo volume and CRL (R^2 = 0.60). The new semi-automated 3-D technique provides reliable measures of embryo volume. Further work is required to assess the validity of this technique. (E-mail: shyamaly@doctors.org.uk)

Key Words: **Embryo volume, SonoAVC, VOCAL, Reliability.**

INTRODUCTION

Growth of the embryo during the first trimester is an important predictor of pregnancy outcome, early growth restriction being predictive of miscarriage (Mantoni and Pedersen 1982; Mukri et al. 2008; Reljic 2001; Smith et al. 1998).The current standard of measuring embryonic growth involves a 2-D measurement of the crown rump length (CRL), which is plotted against the gestational age estimated from the time of the last menstrual period (LMP) (Hadlock et al. 1992; Robinson 1973). Measurement of embryo volume would enable growth to be assessed more critically because it is a nonlinear, exponential phenomenon unlikely to be accurately reflected in a simple 2-D measure of distance.

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Three-dimensional ultrasound facilitates volume measurement of small structures including the embryo. Volume analysis of the embryonic head and trunk can be performed using a computer-generated trapezoid formula including information from a series of thin 2-D slices of the embryonic image taken perpendicular to the midaxial plane (Deurloo et al. 2007; Hafner et al. 2001). Embryo volume can also be calculated in a similar way through the application of Echopac, software that allows the user to manually define the object of interest (Blaas et al 2006). The Echopac technique also allows the user to incorporate the limb buds, which improves the validity of this measurement technique because these structures constitute a substantial proportion of the fetal volume ranging from 5% at 7 weeks' gestation to 10% at 12 weeks' gestation. Echopac is relatively slow, however, taking on average somewhere between 20–30 minutes to perform per embryo. Virtual Organ Computer-aided AnaLysis (VOCAL; GE Healthcare, Zipf, Austria) offers another manual 3-D method for volume calculation and has been used to measure embryo volume (Aviram et al.

2004; Falcon et al. 2005a, 2005b; Martins et al. 2009; Rolo et al. 2009). VOCAL involves the measurement of a series of 2-D images generated as the dataset is rotated about a fixed axis through a number of predetermined steps defined by a rotational angle. Most studies using VOCAL have traced the contours of the fetal head and trunk alone and excluded the limbs, which are technically complex to include (Falcon et al. 2005a, 2005b). One study in which Aviram et al. (2004) did include the fetal limbs did not describe their technique in great detail and used a 30° rotation step, which provides the least number of 2-D planes for the volume calculation and is unlikely to have allowed a structure as irregular as the embryo to have been defined in any detail (Raine-Fenning et al. 2003). The most recent technique for assessment of the embryo, ''virtual embryoscopy,'' involves the holographic projection of the embryo in a ''virtual i-space'' (Verwoerd-Dikkeboom et al. 2008). This display is interactive and the user can examine or measure the embryo using a variety of tools and a ''region growing technique.'' This technique is exciting but currently limited by its expense and the need for specialized equipment.

More recently, semi-automated measurements of 3-D data have become possible. Sonography-based automated volume count (SonoAVC; GE Medical Systems, Zipf, Austria), designed for follicle tracking in assisted reproduction, provides an automated estimation of the relative dimensions and volume of any fluid-filled structure (Raine-Fenning et al. 2008). SonoAVC has been shown to provide valid volumetric measures both *in vivo* (Raine-Fenning et al. 2009) and *in vitro* (Deutch et al. 2008) and helps improve workflow (Jayaprakasan et al. 2008). These studies included measurements of small structures and, in principle, SonoAVC could be used to quantify the volume of the amniotic and extra-amniotic cavities (*i.e.*, the fluid within the gestation sac).

The volume of the embryo can be calculated by subtracting the total volume of all of the fluid within the gestation sac from the volume of the gestation sac as a whole. The fluid within the gestation sac is comprised of that within the amniotic cavity (AC) and the extra-amniotic cavity (EAC), which includes the yolk sac (YSV). The overall volume of the gestation sac can be calculated using VOCAL. The fluid volumes were calculated using a combination of SonoAVC and VOCAL; SonoAVC was used to automatically measure the volume of the AC and EAC, whereas VOCAL was used for the yolk sac because the latter is not always hypoechoic on ultrasound.

The software also has the potential to address the issues surrounding the irregular embryonic contour, assessment of the limb buds and the physiological process of midgut herniation without compromising the time required for measurement of the embryo, by virtue of the semi-automated nature of SonoAVC, which enables the volume of the region-of-interest to be calculated without the need for the time-consuming process of manual delineation.

This study was designed to assess the feasibility and intraobserver reliability of semi-automated measurements of embryo volume made using a combination of VOCAL and SonoAVC. We hypothesized that this technique would provide highly reliable measures of embryo volume and that these would correlate with measures of the embryonic CRL.

METHODS

Experimental design

We aimed to prospectively recruit 46 live, singleton intrauterine pregnancies conceived after IVF treatment. This value was determined according to the method described by Walters *et al.* (1998), based on acceptance of a 95% confidence interval for intraclass correlation lying between 0.8 and 0.9 as being indicative of ''a high degree of reliability'' and in the context of two serial measurements of the same subject. Subjects had to be between 5 and 10 weeks' gestation as determined by the date of oocyte retrieval. Pregnancies without an identifiable fetal pole and those embryos without cardiac heart activity were excluded. We planned to continue recruitment until these 46 subjects had been identified and fetal viability was confirmed.

The study was conducted in accordance with the ethical principles found in the Declaration of Helsinki (1996), the principles of Good Clinical Practice and the Department of Health Research Governance Framework for Health and Social Care (2005). The study was approved by the National Health Service (NHS) research ethics committee, and informed, written consent was obtained before the enrollment of each subject.

Data acquisition and measurement

A baseline 2-D transabdominal and transvaginal ultrasound examination were performed in all subjects in accordance with the Standard Operating Protocol in the assisted conception unit for assessment of early pregnancy. If the presence of a single embryo with cardiac activity was confirmed, a 3-D transvaginal ultrasound examination was then performed. A Voluson E8 Expert (General Electric Medical Systems) ultrasound machine and a RIC 5-9MHz transvaginal probe with a 179° scan angle were used for all examinations. Although the focal zone and magnification were adjusted on a case-by-case basis to ensure the best 2-D image was obtained, all other settings were maintained throughout the study: harmonic frequency low, power 100, gain 1, speckle reduction imaging 3, dynamic contrast 7 and a single focal zone.