Introduction

Normal pregnancy outcome is dependent on optimal placental development and vasculature. Without question real-time evaluation of placental pathology will improve diagnosis and close monitoring of high-risk pregnancies.[1] In recent years there have been significant developments in the use of 3 dimensional (3D) Power Doppler imaging and quantitative 3D Power Doppler (3DPD) histogram analysis to estimate both placental volume and intra-placental vasculature. The vascularisation index (VI) is an indicator of overall perfusion or vascularity, the flow index (FI) measures the intensity of blood flow at the time of the 3D sweep and the vascularisation-flow index (VFI) represents fractional moving blood volume. Reports in the literature suggest that placental volume advances steadily as gestation increases, however there are conflicting results relating to the changes in placental vasculature with gestation.[2-5]

The placenta normally matures and calcifies as the fetus approaches term and from 40 weeks gestation approximately 20% of placentas have extensive calcification.[6]. Calcification occurring at an earlier stage, however, has been associated with pregnancy induced hypertension, fetal growth restriction, and intra-partum fetal distress. Abnormal placental calcification is also thought to be associated with diabetes and Rh incompatibility, with delayed placental maturation occurring in these conditions.[7] Currently ultrasound assessment of placental calcification relies on Grannum grading, which is based on a subjective observation of the placenta following which a grade of 0, I, 11 or 111 is given depending on both the presence and extent of calcification which is present.[8] Many clinicians suspect that Grannum grading as a method of assessing calcification is not reliable, mainly due to its possible subjectivity and poor reproducibility.[9-11] However to date no other ultrasound method has been put forward as an alternative.

Further research into 3D ultrasound and alternative methods of placental assessment is vital if progress is to be made in optimising placental functional assessment as part of the evaluation of fetal health. This study sets out to determine if placental volume, vascularisation and blood flow are correlated with gestational age, with a view to establishing values for normal placental volume and indices of vasculature with gestation. It also examines whether or not a new software method for analysis of percentage calcification (the 'placentometer') correlates well with gestation, in normal pregnancy.

Materials and methods

This was a prospective cohort study. Ethical approval for the study was granted by the Ethics Research Committee of the National Maternity Hospital, Dublin. With informed, written, maternal consent, two hundred and fifty women were recruited to the study. Criteria for normality were that there had been no pv bleeding at any stage in the pregnancy, that the patient had no medical disorder requiring treatment, e.g. diabetes, or any degree of hypertension. Women with a diagnosis of a fetal anomaly or a suspicion or diagnosis of intrauterine growth restriction were also excluded.

Each woman underwent one scan, gestational age at the time of the scan ranging from 12+6 to 39+5 weeks. All scans were performed using a Voluson 730 Expert ultrasound machine (GE Medical Systems, Austria), equipped with curved array transducers. A 2 to 7MHz transducer was used to acquire all two dimensional (2D) images, and a 4 to 8 MHz transducer was used to acquire the three dimensional (3D) images. Each scan incorporated assessment of placental site, amniotic fluid volume, fetal biometry and estimation of fetal weight (after 30 weeks gestation).

A 3DPD placental image was saved at each scan for subsequent analysis of images to calculate volume, VI, FI and VFI. This was done using the Virtual Organ Computer-aided

AnaLysis (VOCAL^{TM}) software (3 dimensional Sonoview, GE Healthcare). The method for saving and analysing images has been previously described.[5] Following rotation of each image by 180^{O}, in six rotational steps of 30^{O} each, a shell contour was displayed in the lower right hand corner of the display, and the volume automatically calculated. Once the volume was accepted 'Contour Histogram' was selected from the VOCAL menu, which allowed the window with the calculated histogram to appear on the screen, displaying the vascular indices VI, FI and VFI (Figure 1). Placental volume was correlated with gestational age, and for those patients who were scanned between 35 and 40 weeks was correlated with birth weight.

To calculate the percentage of calcification, using the placentometer, the region of interest (ROI) was selected, by drawing an outline around the placenta using a pointing device controlled by the mouse. Pixels were recorded following the mouse movements, and then joined into line-segments. These segments were combined to form a continuous outline. The ROI included the basal, body and surface areas of the placenta (Figure 2). A slider was then used to alter the intensity threshold for defining calcification within the ROI. A flood-filing algorithm created a secondary reference map that is used in a quantification algorithm. Once all the relevant areas of calcification were highlighted selection of the 'Quantify' function allowed the application of metric analysis. An output metric was then produced in the form of pixel counts and the overall percentage of calcification in reference to the total number of pixels within the ROI (Figure 3).

Statistical analysis was performed using PASW statistics, Version 18 (SPSS Inc., Chicago, IL, USA). Placental calcification results were transformed using logarithmic transformation to achieve normal distribution prior to analysis. Linear regression analysis was conducted to determine the relationship between the placental study parameters and gestational age, and to assess statistical significance for a relationship between volume and birth weight. $P<0.05$ was considered statistically significant.

Results

The gestational age (GA) at the time of the scans was divided into the four categories of 10-20 weeks (n=32), 20-30 weeks (n=58), 30-35 weeks (n=75) and 35-40 (n=85). The relationship between variables and GA was predominantly assessed with the gestational age coded as a continuous measure. Placental volume ranged from 33.51cm^3 to 574.73cm^3 and had a mean of 199.81cm^3 (SD 105.42). Placental volume was found to be significantly correlated with gestational age (Figure 4), with an increase of 0.623 per day of gestational age increase (P<0.001). The normal regression equation at this gestation is 3285gms + (1.28 x volume). Placental volume (calculated for scans performed between 35 and 40 weeks gestation) and birth weight were related (P=0.001).

Whilst demonstrating a relative increase per day of gestational age, the three indices representing intra-placental vasculature were found to be independent of gestation (VI +0.012% per day, P=0.199; FI +0.009 per day, P=0.229 and VFI +0.003 per day, P=0.557). The vascularisation index ranged from 3.30 to 46.46 and had a mean of 17.33 (SD 7.05). Flow index ranged from 34.84 to 67.77 with a mean of 49.92 (SD 6.18) and the vascularisation-flow index ranged from 1.28 to 23.28. Mean of VFI was 8.74 (SD 3.85).

The percentage of calcification, as quantified using the placentometer, ranged from 0.00 to 20.94% and had a mean of 2.18 (SD 3.33). Calcification was found to be significantly correlated with gestational age, with an increase of 0.32% per day of gestational age increase (P<0.001). Figure 5 depicts placental calcification values across gestation.

Centile charts were devised based on the values for both 3D placental volume and the percentage of calcification (Table 1).

Discussion

The results of this study show that placental volume is correlated with gestational age, increasing as gestation advances. The equation for calculating expected placental volume is [66.67 + 0.62 per day of GA]. This formula for calculating expected placental volume differs to that put forward in a previous study in 2009, which was [-64.68 + 12.31 x GA).[5] The 2009 study was slightly larger (n=295), and the gestations at which the scans were performed was evenly distributed throughout the GA range (12-40 weeks), which may in part explain this difference. It has long been recognised that a placenta which is larger than normal for gestational age is associated with poor perinatal outcome as a result of preterm delivery, intrauterine growth restriction, chromosomal abnormalities, anaemia, infection and gestational diabetes.[12,13] A large placenta is also associated with an increased risk of adult hypertension.[14] A placenta which is smaller than normal for gestation has also been shown to have an association with high-risk pregnancies, including intrauterine growth restriction, chromosomal anomalies, severe intrauterine infection, and pre-conceptual diabetes mellitus and stillbirth.[15] However with 2D ultrasound placental size is difficult to determine. This study agrees with other recent studies that 3D determination of placental volume holds great promise in improving detection rates of those placentas which are both larger and smaller than expected for gestation.

This study does not show a correlation between the vascular indices and gestation. The FI, which has been shown to have the lowest intra-placental variability of the 3 indices,[4] appears to remain relatively static from 12+6 to 40 weeks gestation. The results highlighted in this study differ from a number of previous studies. In one study it was determined that FI increases with gestation, however in that study gestational age was confined to between 26 and 35 weeks.[16] Another study determined that placental blood flow increased with

gestational age and from their study defined normal vascular indices. However, whilst this study included 199 women, gestational age range was confined to between 14 and 25 weeks.[3] This is important as it has been shown that all three indices have extremely poor reproducibility as gestation increases, due to the fact that it becomes increasingly difficult, if not impossible, to image the entire placenta.[17] Another study which found the vascular indices to increase over time, with significant correlation with gestational age, included 100 women with normal, healthy pregnancies, and the gestational age ranged from 12 to 40 weeks.[2] However, only one third of cases were scanned between 30 and 40 weeks gestation, which is the more difficult period for obtaining a view of the entire placenta.

The findings in this study, on the other hand, agrees with other researchers in this field who also found that the placental vascular indices remained constant throughout gestation, suggesting that vascularisation of the placenta increases in proportion to the increase in volume (and that the vascular indices are independent of placental position).[4,5]

The differences in results may also be partly attributed to the machine settings used. Whilst the FI appears to show the least variability, more recently it is being recognised that further studies are required to standardise the machine settings when obtaining images for vascular indices.[17,18]

This study also looked at placental calcification, with the percentage of calcification evaluated using software analysis (placentometer). The results of the software analysis of calcification are very encouraging as they show that the percentage of calcification, defined by the placentometer, correlates with gestational age, increasing as gestation advances. This suggests that assessment of placental calcification, using software analysis has the potential to become an alternative method to Grannum grading.

Conclusion

In conclusion, in normal pregnancies, placental volume appears to increase with gestational age, whereas vascularisation and blood flow appear to be independent of gestation. Placental volume, calculated between 35 and 40 weeks gestation, is related to birth weight. Software analysis of the percentage of calcification, using the 'placentometer' demonstrates the expected increase in calcification with increasing gestational age.

References

1. Konje JC, Huppertz B, Bell SC, Taylor DJ, Kaufmann P. 3-dimensional colour power angiography for staging human placental development. *Lancet* 2003;**362**:1199-1201.

2. Yu CH, Chang CH, Ko HC, Chen WC, Chang FM. Assessment of Placental Fractional Moving Bood Volume using Quantitative Three-Dimensional Power Doppler Ultrasound. *Ultrasound Med Biol* 2003;**29**(1):19-23.

3. Zalud I, Shaha S. Evaluation of the utero-placental circulation by three-dimensional Doppler ultrasound in the second trimester of normal pregnancy. *J Matern Fetal Neonatal Med* 2007:**20**(4):299-305.

4. Guiot C, Gaglioti P, Oberto M, Piccoli E, Rosato R, Todros T. Is three-dimensional power Doppler ultrasound useful in the assessment of placental perfusion in normal and growth-restricted pregnancies?' *Ultrasound Obstet Gynecol*, 2008;**31**:171-6.

5. de Paula CFS, Ruano R, Campos JADB, Zugaib M. Quantitative Analysis of Placental Vasculature by Three-Dimensional Power Doppler Ultrasonography in Normal Pregnancies From 12 to 40 Weeks of Gestation. *Placenta* 2009;**30**:142-8.

6. Abramowicz JS, Sheiner E. Ultrasound of the placenta: A systematic approach. Part 1 Imaging. *Placenta* 2008;**29**:225-40.

7. Clair M, Rosenberg E, Tempkin D, Andreotti RF, Bowie JD. Placental grading in the complicated or high-risk pregnancy. *J Ultrasound Med* 1983;**2**:297-301

8. Grannum PAT, Berkowitz RL, Hobbind JC. The ultrasonic changes in the maturing placenta and their relation to fetal pulmonic maturity. *Am J Obstet Gynecol* 1979;**133**:915-22.

9. Bricker l, Neilson JP, Dowsell T. Routine ultrasound in late pregnancy (after 24 weeks' gestation). *Cochrane Database of Systematic Reviews* 2008;**4.** Art No: CD001451. DOI: 10.1002/14651858.CD001451.pub3.

10. Sau A, Seed P, Langford K. Intraobserver and interobserver variation in the sonographic grading of placental maturity. *Ultrasound Obstet Gynecol* 2003;**23**(4):374-7.

11. Moran M, Ryan J, Higgins M, Brennan PC, McAuliffe FM. Poor agreement between operators on grading of the placenta. J Obstet Gynaecol 2011;**31**(1):24-8.

12. Dombrowski MP, Wolfe HM, Saleh A, Evans MI, O;Brien J. The sonographically thick placenta: a predictor of increased perinatal morbidity and mortality. *Ultrasound Obstet Gynecol* 1992:**2:**252-5.

13. Jauniaux E, Nicolaides K, Hustin J. Perinatal Features Associated with Placental Mesenchymal Dysplasia. *Placenta*, 1997:**18:**701-6.

14. Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995;**311**:171-4.

15. Kidron D, Bernheim J, Aviram R. Placental Findings Contributing to Fetal Death, a Study of 120 Stillbirths between 23 and 40 Weeks Gestation. *Placenta* 2009;**30**:700-4.

16. Filho HAG, Araujo Junior E, Matter R, Da Costa LLD, De Mello Junior CF, Nardozza LMM et al. Placental blood flow measured by three-dimensional power Doppler ultrasound at 26-35 weeks gestation in normal pregnancies. *J Matern Fetal Neonatal Med* 2010;**23**(1):69-73.

17. Lai PK, Wang YA, Welsh AW. Reproducibility of regional placental vascularity/perfusion measurement using 3D power Doppler. *Ultrasound Obstet Gynecol*, 2010;**36**:202-9.

18. Welsh A. The questionable value of VOCAL indices of perfusion. *Ultrasound Obstet Gynecol* 2010;**36**:126-30.