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Title: Multiple prenatal ultrasound scans and ocular development: 20-year follow-up of a randomised, controlled trial

Short Title: Multiple prenatal ultrasound scans and ocular development

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ABSTRACT

Objectives: Through comprehensive ophthalmic examination of adult offspring we sought to determine the impact of multiple prenatal ultrasound scans on ocular development.

Methods: 2743 pregnant women recruited to the Western Australian Pregnancy (Raine) Cohort study during 1989-1991 were randomised to receive either multiple prenatal ultrasounds and Doppler flow studies (intensive group) or single ultrasound at 18 weeks' gestation at King Edward Memorial Hospital, Western Australia. Neonatal birth weight of offspring and other physical measurements were collected prospectively. At age 20 years, participants underwent a comprehensive ophthalmic examination including measures of ocular biometry and visual acuity.

Results: Complete data were available for 1134 adult offspring participants. The mothers of 563 of these had been randomised to receive multiple prenatal ultrasounds. The mean age of participants at follow-up was 20.0 years. On an intention-to-treat basis, except slightly higher intraocular pressures identified in individuals exposed to multiple ultrasound, there was no statistically significant difference between the two groups with regard to ocular biometric or visual outcomes. Although infants in the intensive-ultrasound arm were more likely to have birth weights in the lower quartiles, this was not reflected in adult eye development. Axial length, lens thickness, corneal curvature and thickness and

optic-disc-to-cup ratio were not significantly influenced by the more frequent ultrasound protocol.

Conclusions: Prior to this study, there was a paucity of ultrasound safety data for eye development. We found that frequent in utero ultrasound, including B-mode imaging and use of spectral Doppler mode, from 18 weeks' gestation had no measurable impact on visual outcomes or ocular biometry.

INTRODUCTION

Fetal ultrasound imaging has greatly improved prenatal care by allowing for early identification of developmental problems. Ultrasound is perceived as the safest imaging modality; however, studies have shown at least subtle neurological effects in the offspring including non-right-handedness.¹ Very early findings of delayed speech and dyslexia have not been replicated in well designed studies.²⁻⁴

Sonographers have reported apprehension when performing fetal ultrasound as crystalline lenses are hyperechoic and questions have been raised as to whether ultrasound can influence ocular development. Ultrasound may cause thermal and cavitation effects in tissue and these effects increase with power output.⁵ In the developing eye the dose required to cause thermal cataract is unknown. The developing lens is particularly susceptible to intrauterine insult, such as Rubella infection, but despite large epidemiological studies, the causes of the majority of congenital/infantile cataracts remain unknown.⁶

A previous study performed in Norway during the early 1990s to investigate the relationship between routine ultrasonography and subsequent reduced vision in children randomised participants to ultrasound screening at 19 and 32 weeks' gestation or not.⁷ This study reported no difference in visual acuity or strabismus in the screened or control group at 7 years of age. The Norwegian study is limited as it assessed visual acuity in participants at an age when eye development was not complete and no biometry measures were assessed. Furthermore, measuring visual acuity in young children is difficult and can be unreliable.

The aim of this study was to determine the effect of frequent prenatal ultrasound on eye development using a prospective pregnancy cohort, which had been recruited approximately 20 years ago. The initial investigators hypothesised that more intensive ultrasound scanning would improve pregnancy outcomes for this group. The initial randomised, controlled trial (RCT) found that babies in the intensive-ultrasound arm were more likely to be growth restricted, with increased numbers of newborns with birth weights in the lower percentiles.⁸ Given the size difference in the babies, we hypothesised a potential risk for nanophthalmia (congenitally small eye size) and hypermetropia (longsightedness) in the intensive group. Moreover, an adverse effect of frequent ultrasound on eye development is plausible given the subtle neurological effects seen in human studies and the thermal and cavitation effects on cells seen in animal models. Furthermore, the eye is easily accessible to make precise measurements not possible elsewhere in the neurological system. At the age 20, participants underwent full ophthalmic assessment.⁹ In this study, we aimed to determine any detectable impact of the frequent ultrasound protocol on eye development of these young adults.

METHODS

Participants

The Western Australian Pregnancy Cohort (Raine) Study is a prospective, longitudinal pregnancy cohort of 2,868 live births recruited at 16-18 weeks' gestation through King Edward Memorial Hospital, Western Australia, from 1989-1991. Pregnant women were randomised to either the "regular" group receiving

one ultrasound scan at 18 weeks' gestation or the "intensive" group receiving ultrasound imaging and Doppler flow studies at 18, 24, 28, 34 and 38 weeks' gestation.

Complete details of enrolment methods have been described previously.⁸ In brief, inclusion criteria were pregnant women with sufficient English language skills and with an expectation to reside in Western Australia to allow future follow-up. Ninety percent of eligible women agreed to participate in the study and informed consent was gained from the mother at enrolment and from the young adult participant at follow-up. This research adhered to the Declaration of Helsinki and the Human Ethics committee at KEMH approved the ultrasound study protocol.

After 20 years, 1134 of the original 2868 live births completed the 20-year ocular examination with a full data set available. The participation rate of the two groups after 20 years is presented in figure 1. The Human Ethics Committee at the University of Western Australia approved the ocular examination protocol.

Early Life Data

Extensive data were collected on the pregnant women including maternal and family history, prenatal measurements and obstetric information. The total number of scans was recorded. Ultrasound scans were performed using a General Electric 3600 machine with 3.5MHz linear array and 5MHz sector transducer. Measurements were made of the fetal biparietal diameter, occipitofrontal diameter, head circumference, abdominal circumference and femur length at each

visit. Each measurement was taken three times and the average used. Amniotic fluid volume and index and placental morphology and location were also described. Women in the intensive group also had Doppler flow-velocity waveform studies performed with a spectrum analyser (Medasonics SP25A) and a D10 bi-directional continuous wave Doppler system (total power output 3mW, spatial-peak temporal-average 25 mW/cm²).

Ocular Examination

As part of a comprehensive ophthalmic exam of the adult offspring, LogMAR visual acuity, axial length measurement (Zeiss IOL master V.5, Carl Zeiss Meditec AG, Jena, Germany) and cycloplegic autorefraction (Nidek 510 ARK, NIDEK Co.Ltd, Japan) measurements were recorded. Refractive error as measured by mean spherical equivalence was determined by summing the spherical error and half the cylindrical error.

An Oculus Pentacam (Oculus Optikgerate GmbH, Wetzlar, Germany) was used to measure central corneal thickness and perform Scheimpflug imaging for lens thickness. Ocular dominance was assessed by asking participants to look at a distant target through a triangle made by their two hands (the Miles test). The eye the participant used to look through the triangle was recorded as the dominant eye. Amblyopia was determined by comparing the difference in best-corrected visual acuity between the two eyes, after ruling out impairment secondary to primary visual pathway pathology.

Statistical Analysis

There was no statistically significant difference between right and left eye measurements and results are presented for participant's right eyes only. All data were analysed on an intention-to-treat basis. Pre-hoc power calculation showed that a sample size of 145 participants would have a statistical power of 0.95 to detect a 2-letter difference in vision between the groups ($\alpha = 0.05$).

Student's t-test was used for parametric data comparisons whilst the Mann-Whitney U test for non-normally distributed outcomes was used to test the hypothesis that participants in the intensive group would have the same ocular biometry and visual function as those in the control group. For each ocular measure, the standardised mean difference (SMD) was calculated as the mean difference between the two groups divided by the standard deviation for all participants pooled across both groups. Categorical data were analysed using χ^2 test. All statistical analysis was performed using R version 3.0.1 (<http://www.r-project.org/>).

RESULTS

A total of 1344 offspring from the original study attended the ophthalmic follow-up by March 2012. 652 (48.5%) of these participants were female. Participants were aged between 19 and 21 years at follow-up. Study participant characteristics are displayed in Table 1. A comparison of participants included in this study with those who were part of the original RCT cohort was performed using the data from the year 1 follow-up to examine the sociodemographic characteristics between the two groups. These characteristics included family structure (sole parents vs couple families), income levels and SEIFA index of relative advantage and disadvantage of parents/carers. Slightly higher number of participants who attended this follow-up study was born into couple families (89% vs 86%) and families with combined income of more than AUD\$ 25,000 (63% vs 51%). Similarly the mean SEIFA index of relative socio-economic advantage was higher for parents/carers of the recent

cohort (1031±89) compared to parents/carers of their peers who were not examined (1005±86, $p<0.001$).

There was no statistically significant difference in ocular dominance between the two groups ($p=0.62$). In the regular-ultrasound group, 359 (62.9%) were right-eye dominant and 212 (37.1%) were left-eye dominant. In the intensive group 363 (65.5%) of participants were right-eye dominant.

The median visual acuity was -0.06 (IQR: -10.0-0) logMAR score in both regular and intensive groups ($p=0.90$). No groups had a higher inter-ocular difference in visual acuity ($p=0.87$). The refractive error in both regular and intensive groups was non-normally distributed (Shapiro-Wilks normality test, $p<0.001$) with median spherical equivalent +0.38 D (inter-quartile range (IQR): -0.38, +0.63) in the regular group and +0.25 D (IQR: -0.38, +0.63) in the intensive group ($p=0.30$) (Table 2).

None of the anterior biometric measures were significantly different between the two groups (Table 3). While no optic disc parameter was different, slightly higher intraocular pressure was detected in the intensive-ultrasound group ($p=0.034$) (Table 4).

DISCUSSION

This study presents the first RCT measuring the biometric and functional effects of pre-natal ultrasound scans at an age when eye development is complete and

participants could reliably complete the required examinations. Overall we found no detrimental consequence of multiple ultrasound scans on ocular health. This study confirms the findings of the Norwegian study which examined vision in children who had been exposed to two ultrasounds.⁷ While the initial Raine RCT found a significant difference in rates of intrauterine growth restriction, the difference had disappeared by one year of age.¹⁰ No other RCT of prenatal ultrasound scans has replicated this effect on growth, although there have not been other studies with this study design. There was no difference in physical size at 2- or 8-year follow-up of the Raine participants and no difference in cognitive function, behaviour and language development at 8 years.⁴ Given that taller adults have longer axial lengths, the intensive-ultrasound group might be expected to have shorter axial lengths and less myopia; however, axial lengths and refractive errors were similar between the two groups.

Interestingly, a study of over 300 mice revealed that prenatal ultrasound exposure for a total of 30 minutes a day resulted in a statistically significant number of cortical neurons failing to acquire their proper position during neuronal migration.¹¹ While a follow-up study of the initial Raine RCT has found no difference in physical size or standard tests of childhood speech, behaviour and language at 8 years of age, this potential risk to visual pathway development exists.⁴ Reassuringly, the rates of amblyopia (which in some may result from abnormal neuronal development) are similar between the intensive and regular groups of this study.

We did not identify any significant difference in optic disc size between the intensive- and regular-ultrasound group. While a statistically significant difference is present in intraocular pressure between the two groups, we believe this difference has minimal clinical significance. Although the effect size based on SMD was very close to zero suggests that exposure to regular and intensive ultrasounds have equivalent effects on intraocular pressure, further research should be done to investigate slightly higher intraocular pressure observed in individuals exposed to multiple ultrasounds.

At the time the prenatal ultrasounds were performed, acoustic outputs did not exceed a spatial-peak temporal-average intensity of 94mW/cm^2 . However current limits in the United States allow a spatial-peak temporal-average intensity of 720mW/cm^2 for fetal applications. The acoustic output from modern ultrasound scanners has increased 10-15 times during the last decade. While individual scans may be of short duration, cumulative exposure time may be an issue in risk assessment.⁵ The other limitation of the study design is that the control group was exposed to one ultrasound. Designing an epidemiological study of ultrasound safety is thus very difficult given that virtually every pregnant woman now undergoes at least one screening ultrasound. The As Low As Reasonable Achievable (ALARA) principle should be adhered to for ultrasound outputs and we must rely on the findings of studies such as this one that have demonstrated ultrasound safety.

The main strength of this study is that the prospective RCT design eliminates the bias that might occur due to increased ultrasound usage and poor visual outcomes. Furthermore, participants underwent a comprehensive 2.5-hour eye examination so very accurate measurements were made and it was unlikely that any pathology was missed. This long-term follow-up was performed at an age when eye development is complete; however, participants remain free of confounding diseases.

Exposure to multiple prenatal ultrasounds from 18 weeks' gestation does not impair eye development as measured by visual acuity and detailed ocular biometry.

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FIGURES AND TABLES

Figure 1 Randomization, allocation, follow-up and analysis

Table 1: Study participant characteristics

	Regular Ultrasound (n=571)	Intensive Ultrasound (n=563)
Gender (% Males)	297 (52%)	296 (52%)
Mean age in years ($\pm$ SD)	19.9 ($\pm$ 0.37)	20.1 ($\pm$ 0.44)
Number of Ultrasounds (range)	1 (1)	6 (1-10)
Mean birth weight (g)	3362($\pm$ 558)	3306($\pm$ 573)
Number of participants with birth weight		
<75 th centile	416	433
<50 th centile	286	277
<25 th centile	136	147

Table 2: Comparison of vision and refractive error between regular and intensive ultrasound groups (Median (Interquartile Range)).

Ocular Measure	Regular Ultrasound	Intensive Ultrasound	p-value	Standardised mean difference (95%CI)
Best-Corrected VA (logMAR)	-0.06 (-0.10, 0)	-0.06 (-0.10, 0)	0.895	0.001 (-0.115 to 0.117)
Inter-ocular Difference in VA (Amblyopia)	0.04 (0.02, 0.08)	0.04 (0.02, 0.08)	0.753	0.036 (-0.081 to 0.152)
Spherical Equivalent (D)	+0.38 (-0.38, +0.63)	+0.25 (-0.38, +0.63)	0.304	-0.801 (-0.922 to -0.680)
Astigmatism (D)	-0.25 (-0.5, -0.25)	-0.25 (-0.5, -0.25)	0.222	-0.098 (-0.215 to 0.018)

Table 3: Anterior ocular biometry of the regular vs intensive ultrasound groups (Mean $\pm$ SD).

Ocular Measure	Regular Ultrasound	Intensive Ultrasound	p-value	Standardised mean difference (95%CI)
Central Corneal Thickness (mm)	538 $\pm$ 33	538 $\pm$ 32	0.806	-0.001 (-0.117 to 0.115)
Corneal Curvature (mm)	43.65 $\pm$ 1.40	43.66 $\pm$ 1.45	0.892	0.0003 (-0.116 to 0.117)
Lens Thickness (mm)	3.47 $\pm$ 0.28	3.48 $\pm$ 0.24	0.539	0.003 (-0.114 to 0.119)
Anterior Chamber Depth (mm)	3.71 $\pm$ 0.27	3.72 $\pm$ 0.27	0.391	0.004 (-0.113 to 0.120)
Axial Length (mm)	23.60 $\pm$ 0.91	23.63 $\pm$ 0.97	0.503	0.002 (-0.115 to 0.118)

Table 4: Comparison of optic disc measures and intraocular pressures.

Ocular Measure	Regular Ultrasound	Intensive Ultrasound	p-value	Standardised mean difference (95%CI)
Optic Disc Area (mm ²)	1.85 (1.56, 2.21)	1.87 (1.55, 2.27)	0.380	0.017 (-0.099 to 0.134)
Optic Rim Area (mm ²)	1.45 (1.26, 1.70)	1.45 (1.26, 1.68)	0.992	0.011 (-0.106 to 0.127)
Optic Cup to Disc Ratio	0.19 ± 0.12	0.20 ± 0.12	0.207	0.045 (-0.071 to 0.161)
Intraocular Pressure (mmHg)	15.37 ± 3.22	15.79 ± 3.49	0.034	0.059 (-0.089 to 0.144)

REFERENCES

1. Salvesen KA. Ultrasound in pregnancy and non-right handedness: meta-analysis of randomized trials. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2011;38:267-71.
2. Salvesen KA, Vatten LJ, Bakketeig LS, Eik-Nes SH. Routine ultrasonography in utero and speech development. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 1994;4:101-3.
3. Salvesen KA, Bakketeig LS, Eik-nes SH, Undheim JO, Okland O. Routine ultrasonography in utero and school performance at age 8-9 years. *Lancet*. 1992;339:85-9.
4. Newnham JP, Doherty DA, Kendall GE, Zubrick SR, Landau LL, Stanley FJ. Effects of repeated prenatal ultrasound examinations on childhood outcome up to 8 years of age: follow-up of a randomised controlled trial. *Lancet*. 2004;364:2038-44.
5. Abramowicz JS, Barnett SB, Duck FA, Edmonds PD, Hynynen KH, Ziskin MC. Fetal thermal effects of diagnostic ultrasound. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine*. 2008;27:541-59; quiz 60-3.
6. Haargaard B, Wohlfahrt J, Rosenberg T, Fledelius HC, Melbye M. Risk factors for idiopathic congenital/infantile cataract. *Invest Ophthalmol Vis Sci*. 2005;46:3067-73.
7. Salvesen KA, Vatten LJ, Jacobsen G, et al. Routine ultrasonography in utero and subsequent vision and hearing at primary school age. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 1992;2:243-4, 5-7.
8. Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet*. 1993;342:887-91.
9. Yazar S, Forward H, McKnight CM, et al. Raine eye health study: design, methodology and baseline prevalence of ophthalmic disease in a birth-cohort study of young adults. *Ophthalmic Genet*. 2013;34:199-208.
10. Macdonald W, Newnham J, Gurrin L, Evans S. Effect of frequent prenatal ultrasound on birthweight: follow up at 1 year of age. Western Australian Pregnancy Cohort (Raine) Working Group. *Lancet*. 1996;348:482.
11. Ang ES, Jr., Gluncic V, Duque A, Schafer ME, Rakic P. Prenatal exposure to ultrasound waves impacts neuronal migration in mice. *Proc Natl Acad Sci U S A*. 2006;103:12903-10.