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Adrenal gland length in euploid and trisomy 18 fetuses at 11–13 weeks

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Objectives To establish a normal range for fetal adrenal gland length at 11–13 weeks' gestation and to investigate whether the length is altered in fetal trisomy 18.

Methods Fetal adrenal gland length was measured by three-dimensional ultrasound in fetuses at low risk of aneuploidies ($n = 400$) and another group at high risk, including 380 euploid fetuses and 41 with trisomy 18. The data of the low-risk group were used to establish a reference range of adrenal gland length with crown–rump length (CRL). In the high-risk group, adrenal gland length in the euploid and trisomy 18 groups was compared.

Results In the low-risk group, fetal adrenal gland size increased exponentially with fetal CRL from a median of 2.3 mm at CRL of 45 mm to about 4.4 cm at CRL of 84 mm. In trisomy 18, the median adrenal gland length for CRL was significantly lower than the median in the low-risk group (−1.37 mm; interquartile range: −1.67 to −0.99 mm, $p < 0.0001$). In euploid fetuses, the adrenal gland size was not significantly from the low-risk group ($p = 0.100$).

Conclusion Trisomy 18 is associated with adrenal gland hypoplasia which is apparent at 11–13 weeks' gestation.

KEY WORDS: first trimester screening; adrenal gland; trisomy 18; nuchal translucency

INTRODUCTION

The fetal adrenal glands are the main source of estrogens during pregnancy and are crucial for normal fetal development (Rainey *et al*., 2004). The fetal adrenal gland is composed of an outer neocortex involved in the production of aldosterone, an intermediate transitional zone which produces cortisol and an inner fetal zone which accounts for 85% of the glandular mass, and produces the steroid precursor dehydroepiandrosterone-sulfate (DHEA-S). This precursor is used by the placenta for the production of estradiol or is first converted to 16OH-DHEAS in the fetal liver and then to estriol in the placenta.

Early attempts at prenatal assessment of the fetal adrenal glands utilized transabdominal two-dimensional (2D) ultrasound and reported that the glands could be consistently seen after 30 weeks but rarely before 20 weeks (Lewis *et al*., 1982; Rosenberg *et al*., 1982; Jeanty *et al*., 1984; Hata *et al*., 1985). Subsequently, a study utilizing transvaginal 2D ultrasound to examine 100 normal fetuses at 12–17 weeks reported that the adrenal glands were always visible, and in a coronal view the maximal length of the gland parallel to the fetal spine was measured (Bronshtein *et al*., 1993). The adrenal gland length increased linearly with gestation from a mean of 2.5 mm at 13 weeks to 4.5 mm at 17 weeks.

In fetuses with trisomy 18, the adrenal gland may be hypoplastic. Postmortem studies have reported that in fetal trisomy 18, the weight of the adrenal glands is substantially lower than in normal fetuses (Barr, 1994). Additionally, in trisomy 18 pregnancies the maternal serum concentration of estriol is significantly lower than in normal pregnancies both during the second- and in the first-trimester of pregnancy (Aitken *et al*., 1993; Crossley *et al*., 1993; Yankowitz *et al*., 1998).

The aims of this study are to use 3D ultrasound for accurate definition of the fetal adrenal gland length at 11–13 weeks' gestation and investigate whether in fetal trisomy 18 the adrenal length is lower than in normal fetuses.

METHODS

This was an analysis of 3D volumes of the fetal adrenal gland obtained from two groups of singleton pregnancies undergoing screening for aneuploidies between 11 and 13 weeks and 6 days by a combination of maternal age, serum-free β-human chorionic gondadotropin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) and fetal nuchal translucency (NT) thickness (Snijders *et al*., 1998; Kagan *et al*., 2008). Approval for the study was obtained from the hospital Ethics Committee.

The entry criteria for the first group of 400 fetuses were first, estimated risk for trisomy 21 and trisomy

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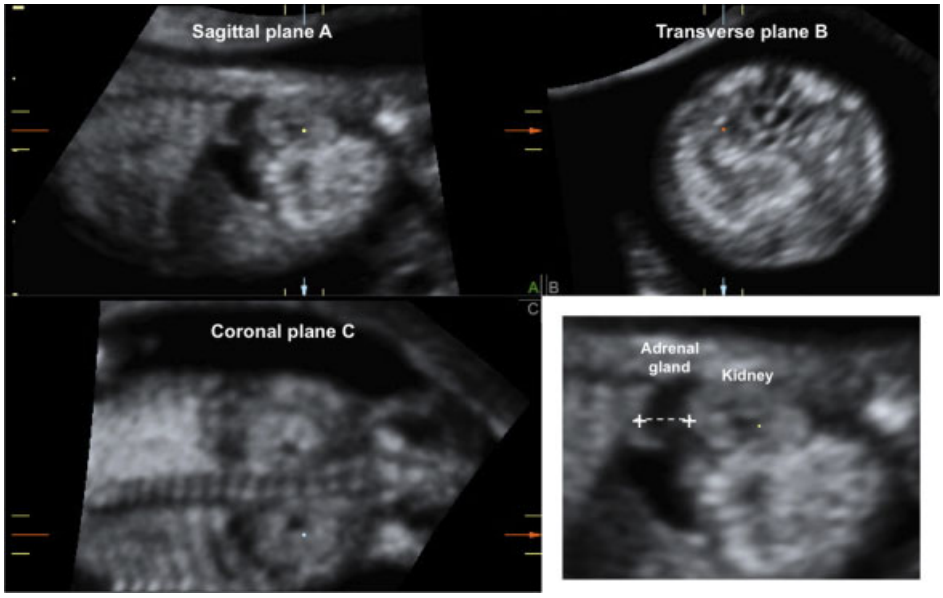

Figure 1—Measurement of the adrenal gland length using three-dimensional ultrasound

18 of <1 in 300 and second, no obvious fetal defects at the 11–13 weeks' and 20–22 weeks' scan (low-risk group). In the second group of fetuses, adrenal gland volumes were obtained within 1 h before chorionic villus sampling (CVS) for karyotyping (pre-CVS group). We subsequently selected all cases of trisomy 18 ($n = 41$) and euploid controls ($n = 380$).

In all cases, a midsagital view of the fetal trunk was obtained by either transabdominal or transvaginal sonography (RAB4-8 and RIC5-9 transducer, Voluson E6; GE Medical Systems, Milwaukee, WI, USA) and a 3D volume of the lower thorax and abdomen was acquired and stored for subsequent analysis by sonographers with extensive experience in 3D ultrasound. In each volume, the multiplanar mode was used to obtain the midsagittal view of the fetus in plane A. This was confirmed to be midsagital by observing a circular transverse section of the abdomen in plane B and the midline position of the spine in the coronal plane C. The image in plane A was then scrolled through a series of left and right parasagittal sections to identify each kidney and adrenal gland (Figure 1). The image was frozen when the maximum length between the top of the kidney and the dome of the adrenal gland was identified and the calipers of the ultrasound machine were used to obtain this measurement (Figure 1).

The agreement and bias for the measurements of adrenal gland size by a single examiner and between two different examiners was investigated from the study of 3D volumes obtained from 25 cases which were selected at random from the study population. One examiner (A) who made the original measurements repeated each measurement and a second examiner (B) made the measurements once. The examiners were not aware of the measurements of each other and examiner A when making the measurements on the second occasion was not aware of measurements on the first occasion.

Statistical analysis

Continuous and categorical variables were compared using Mann–Whitney U-test and χ^2-square test or Fisher's exact test, respectively. The distribution of adrenal gland length was made Gaussian using logarithmic transformation ($\log_{10}$). Normality of distribution was assessed using probability plots and Shapiro–Wilk test ($p = 0.109$).

The Bland–Altman analysis was used to compare the degree of agreement and bias between measurements by a single examiner and two different examiners for adrenal gland length (Bland and Altman, 2003). Paired t-test was used to compare the significance of difference between these paired measurements. The length of the right adrenal gland was bigger than the left and the measurements of the right were more reproducible (see the section on Results) Therefore, in the subsequent analysis we selected the right adrenal gland.

In the low-risk group, multiple regression analysis was used to determine the factors among maternal characteristics and fetal crown–rump length (CRL) that provided significant contribution to the prediction of $\log_{10}$ transformed adrenal gland length. The only factor providing significant contribution was fetal CRL (see the section on Results) and, therefore the measured length in each case in the pre-CVS group was expressed as a difference from the expected normal mean for fetal CRL (delta value). Similarly, the measured NT in each case was expressed as a difference from the expected normal mean for fetal CRL (delta value) (Wright *et al.*, 2008). Multiple regression analysis was then used to determine the significance of contribution to delta adrenal gland length from delta NT and fetal karyotype.

The statistical software packages SPSS 16.0 (SPSS, Chicago, IL, USA) and XLSTAT-Pro 2010 (Addinsoft, New York, USA) were used for data analyses.

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Table 1—Maternal and pregnancy characteristics of the study populations

Characteristics	Low-risk group ($n = 400$)	Chorionic villus sampling groups: Euploid ($n = 380$)	Chorionic villus sampling groups: Trisomy 18 ($n = 41$)
Maternal age in years, median (IQR)	32.0 (28.1–36.0)	35.0 (30.5–38.2)*	38.0 (32.5–41.0)*
Body mass index in kg/m^2, median (IQR)	24.2 (21.6–28.2)	24.5 (22.4–28.0)	26.3 (21.4–28.6)
Crown–rump length in mm, median (IQR)	64.3 (58.2–71.4)	67.3 (60.4–74.2)*	55.7 (52.4–64.9)*
Racial origin			
Caucasian, *n* (%)	324 (81.0)	314 (82.6)	32 (78.0)
African, *n* (%)	42 (10.5)	31 (8.2)	5 (12.2)
South Asian, *n* (%)	19 (4.8)	15 (3.9)	3 (7.3)
East Asian, *n* (%)	10 (2.5)	14 (3.7)	1 (2.4)
Mixed, *n* (%)	5 (1.3)	6 (1.6)	0
Cigarette smoker, *n* (%)	27 (6.8)	40 (10.5)	1 (2.4)
Conception			
Spontaneous, *n* (%)	390 (97.5)	367 (96.6)	38 (92.7)
Ovulation drugs, *n* (%)	10 (2.5)	13 (3.4)*	3 (7.3)

Comparisons between groups (χ^2-test and Fisher's exact test for categorical variables and Mann–Whitney U-test for continuous variables): significance value * $p < 0.05$.
IQR, interquartile range.

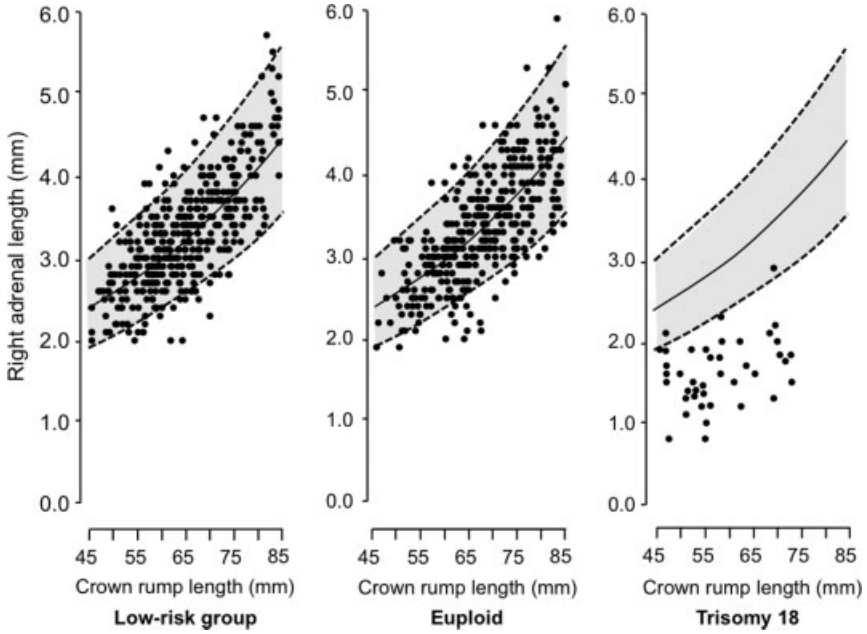

Figure 2—Fetal adrenal gland length with crown–rump length in the low-risk group and in fetuses with normal karyotype and trisomy 18 plotted on the normal rage derived from the low-risk group (shaded area with median, 95th and 5th percentiles)

RESULTS

The maternal and fetal characteristics of the study groups are compared in Table 1. In the low-risk group, regression analysis showed that in the prediction of $\log_{10}$ adrenal gland length, there was a significant contribution from fetal CRL but not from maternal age ($p = 0.349$), body mass index ($p = 0.661$), racial origin ($p = 0.391$), mode of conception ($p = 0.567$) or smoking status ($p = 0.989$).

Expected $\log_{10}$ adrenal gland length $= 0.064 + 0.007 \times$ fetal CRL in mm; SD $= 0.0856$, $R^2 = 0.530$, $p < 0.0001$. In trisomy 18, compared to the low-risk group, delta adrenal gland length was lower and delta NT was higher (Table 2, Figures 2 and 3). In the euploid group compared to the low-risk group, delta adrenal gland length was not significantly different but delta NT was higher. Multiple regression analysis showed that in predicting delta adrenal gland length, there was a significant contribution from fetal karyotype ($p < 0.0001$) but not from delta NT ($p = 0.567$).

The repeatability of measurements of the right and left adrenal gland length by the same and two different examiners are shown in Table 3. In the left adrenal gland length, there was a significant difference in the measurements obtained by the same examiner ($p =$

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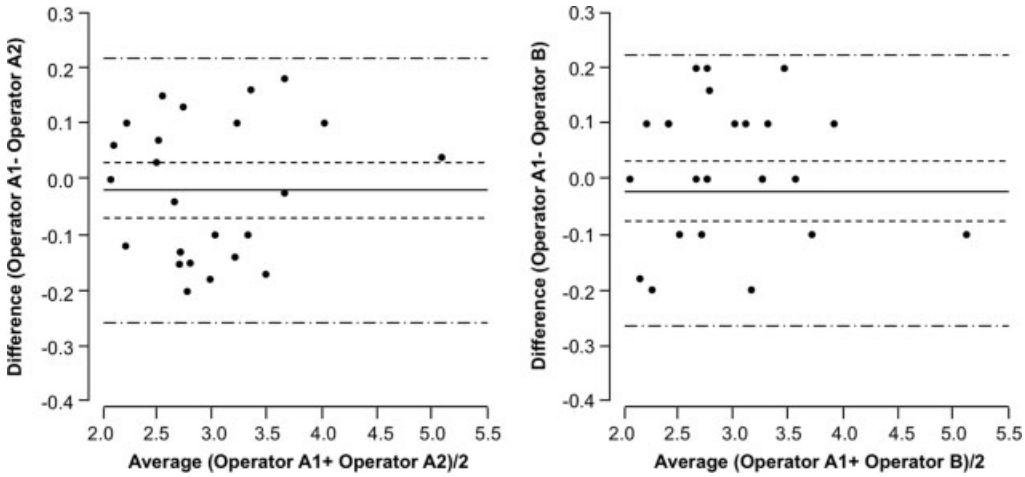

Figure 3—Bland–Altman plots of intra- and inter-observer repeatability in paired measurements of the right adrenal gland length

Table 2—Median and IQR of delta adrenal gland size and delta NT thickness in the study populations

	Delta adrenal gland size Median (IQR)	Delta NT Median (IQR)
Low-risk group	0.00 (−0.32 to 0.25)	0.00 (−0.18 to 0.22)
Chorionic villus sampling group		
Euploid fetus	−0.08 (−0.35 to 0.21)	0.53 (0.13 to 1.17)*
Trisomy 18 fetus	−1.37 (−1.67 to −0.99)*	4.42 (1.87 to 5.87)*

Comparisons between groups by Mann–Whitney *U*-test: $^{*}p < 0.01$.
IQR, interquartile range; NT, nuchal translucency.

Table 3—Bland–Altman analysis for intra- and inter-observer repeatability in paired measurements of the right and left adrenal gland

	Mean difference (95% CI)	Limits of agreement (95% CI)
Right adrenal gland		
Intraobserver variability	−0.020 (−0.071 to 0.031)	−0.261 (−0.312 to −0.312) and 0.221 (0.170 to 0.272)
Interobserver variability	−0.019 (−0.070 to 0.031)	−0.259 (−0.309 to −0.208) and 0.220 (0.170 to 0.270)
Left adrenal gland		
Intraobserver variability	0.364 (0.074 to 0.654)	−1.015 (−1.518 to −0.512) and 1.743 (1.240 to 2.246)
Interobserver variability	0.360 (0.026 to 0.694)	−1.224 (−1.802 to −0.646) and 1.944 (1.366 to 2.522)

CI, confidence interval.

0.016) and those obtained by two different examiners ($p = 0.036$). In contrast, in the right adrenal gland length there was no significant difference in measurements by either the same or by two different examiners ($p = 0.423$ and $p = 0.440$, respectively). The Pearson correlation coefficient between the difference and the mean of measurements by the same examiner and two different examiners for left adrenal gland length were −0.505 [95% confidence interval (CI) −0.751 to −0.138] and −0.455 (95% CI −0.721 to −0.073), respectively and the corresponding measurements for the right adrenal gland were 0.116 (95% CI −0.292 to 0.489) and −0.019 (95% CI −0.379 to 0.411), respectively.

DISCUSSION

The finding of this study show that the fetal adrenal glands can be consistently visualized at 11–13 weeks' gestation and 3D ultrasound can be used to obtain reproducible measurements of adrenal gland length. The length increases exponentially with fetal CRL and is substantially lower in trisomy 18 than in euploid fetuses.

We used 3D ultrasound to ensure through the multiplanar view that the maximum adrenal gland length could be measured in a parasagital plane parallel to the spine. We chose to measure the maximal length rather than the volume of the gland because as a consequence of its small size at this gestation tracing the outline in a series of sections, which is necessary for calculation of volume (Chang *et al.*, 2002), would have inevitably resulted in inaccurate results. The finding of an exponential increase in adrenal gland length with CRL is compatible with the results of a postmortem study which reported that the adrenal gland weight increases exponentially with gestational age from about

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9 mg at 10 weeks to 100 mg at 14 weeks (Carr and Casey, 1982).

In trisomy 18 fetuses at 11–13 weeks' gestation, the adrenal gland length was approximately half of that in normal fetuses of the same CRL and such adrenal hypoplasia could be the underlying cause of the low maternal serum estriol observed in association with this aneuploidy (Aitken *et al*., 1993; Crossley *et al*., 1993; Yankowitz *et al*., 1998). Our results are also consistent with the findings of a postmortem study in fetuses with trisomy 18 at mid-gestation where the average adrenal gland weight to body weight ratio was two standard deviations below the ratio observed in normal fetuses (Barr, 1994). A histological study of three neonatal or infant deaths with trisomy 18 reported that the fetal zone of the adrenal glands, where DHEA-S is produced, was absent or severely hypoplastic (Turner and O'Herlihy, 1984).

At 11–13 weeks' gestation effective screening for trisomies 21, 18 and 13 can be provided by a combination of maternal age, fetal NT thickness, fetal heart rate, maternal serum-free β-hCG and PAPP-A, with estimated detection rates of 91, 97 and 94%, respectively, for an overall false positive rate of 3.1% (Kagan *et al*., 2008). Consequently, 3D assessment of the fetal adrenal, which is a laborious process, is unlikely to be incorporated in routine screening for trisomy 18. However, this study has documented the finding that trisomy 18 is associated with adrenal hypoplasia which is apparent from the first trimester of pregnancy. The possible mechanism underlying such hypoplasia was provided by the results of a recent study which investigated the gene expression profile in amniotic fluid at 17–20 weeks and reported that in pregnancies with fetal trisomy 18 there is downregulation of the genes involved in adrenal gland development (Koide *et al*., 2011).

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