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First-trimester determination of fetal gender by ultrasound: measurement of the ano-genital distance

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ABSTRACT

Introduction: Early ultrasound fetal sex determination is of obvious interest, particularly in the context of X-linked diseases. In the human, the anogenital distance, i.e., the distance between the caudal end and the base of the genital tubercule is sexually dimorphic. This difference is apparent from 11 weeks of gestation.

The aim of this prospective study was to evaluate the accuracy of anogenital distance measurement during the first trimester ultrasound in the early determination of fetal gender.

Materials and methods: Fetal gender was assessed by ultrasound in 310 singleton pregnancies at 11–14 weeks of gestation. The optimal cut-off was determined by the minimal *p*-value technic and validated using bootstrap simulation.

Results: 310 women were included. A cut-off of 4.8 mm was determined to predict male (≥4.8 mm) or female (<4.8 mm) fetuses. Sex was correctly determined for 87% of the males and 89% of the females. The inter-observer variability was excellent.

Conclusion: This study presents a new sonographic sign for early fetal sex determination that has not been previously explored. It appears to be an accurate tool but it requires further validation in larger series.

Introduction

Early fetal sex determination during pregnancy is of great interest for both physicians and future parents for pregnancies at risk of gender-linked genetic disease. To date, fetal sex is determined either by genetic testing or ultrasonography [1].

Genetic tests are highly accurate in determining fetal sex. Chorionic villus sampling under sonography guidance was the first approved technique but is an invasive procedure associated with a risk of pregnancy loss. Analysis of cell-free fetal DNA in maternal blood is a non-invasive technique but expensive and less available worldwide than ultrasonography [2].

The first non-invasive technique to assess gender is based on second trimester fetal ultrasonography with simple morphologic criteria: (i) presence of a penis and scrotum for a male and (ii) labia majora and minora for a female. In the absence of sexual anomalies, this simple and worldwide technique has an accuracy of up to 100% as from 20 weeks of gestation (WG).

In the late nineties, a new method based on first trimester fetal ultrasonography was developed to determine fetal sex earlier. This involved measuring the angle of the genital tubercle to a horizontal line through the lumbosacral skin surface in a mid-sagittal plane with the fetus in a natural position [3,4]. This method gave a 100% sensitivity in fetal sex

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determination after 13 WG but a lower sensitivity between 11 and 12 WG [3,5–8].

Sexual morphogenesis is a dynamic hormono-dependent phenomenon occurring from the sixth WG. Testosterone secretion by testicular cells is responsible for sexual differentiation in the male. Consequently, the anogenital distance (AGD), i.e., the distance between the caudal extremity of the fetus and the base of the genital tubercule, is testosterone dependent and hence sex dependent. The AGD is greater in males than in females [9,10]. In the animal model, it is a marker of fetal exposure to androgens during the masculinization programming window [11,12]. In the human, AGD in male newborns is approximately double that of female newborns. This difference remains significant until 24–30 months of life then decreases up to adulthood [9,10,13]. So far, no study has evaluated the contribution of the ultrasound assessment of AGD between 11 and 13 WG + 6 days (corresponding to the time of the first routine ultrasonography during pregnancy) to determine fetal sex.

Therefore, the aim of the present prospective study was to evaluate whether AGD measurement by ultrasonography between 11 and 13 WG + 6 days could accurately determine fetal sex.

Materials and methods

We conducted a prospective study to evaluate fetal gender by ultrasonography between January and December 2014. AGD was performed by one operator (JSA) in 310 consecutive singleton pregnancies between 11 and 13 WG + 6 days (Crown–rump length (CRL), 45–84 mm) during the routine first trimester ultrasonography.

All patients gave their informed consent to participate in the study. The study protocol was accepted by the Ethics Committee of the *Collège National des Gynécologues et Obstétriciens Français*.

A Voluson E8 Expert HD Live (General Electric Company), equipped with a 4–7-MHz convex transducer was used for all scans. The AGD was evaluated in the mid-sagittal plane with the fetus lying in a natural position (neither hyperflexed nor hyperextended), which is the image used for the CRL measurement. A caudal caliper was positioned as for a CRL measurement and a genital caliper placed at the inferior base of the genital tubercule (Fig. 1).

In the whole population of women, the optimal cut-off for AGD was retrospectively determined by a minimal *p*-value approach. This involved dichotomizing the AGD into dummy variables with a cut-off every 0.1 mm. Chi-square tests comparing the rate of male and female newborns for every dummy variable were then calculated. The cut-off with the minimal *p* value was chosen as the optimal cut-off.

The predictive accuracy of the threshold was assessed by its discrimination. The area under the receiver operating characteristic curve (AUC) measured the threshold's ability to discriminate the sex between patients. An AUC of 0.5 indicates that the model provides no predictive discrimination, while a value of 1.0 indicates perfect discrimination between cases. Measures of predictive accuracy were validated using bootstrap simulation. The threshold was fit to 300 samples of equivalent size drawn at random with replacement from the original study population. The measures of predictive performance obtained for each statistic in the bootstrap samples were used to estimate the bias in the model statistics attributable to overfitting.

Inter-observer agreement was evaluated comparing the measurements of two operators (JSA and JC) on another 50-woman sample. Each operator measured the AGD, blinded to the other operator's measurement and the difference between the two measurements was analysed.

Statistical analysis was based on Student's *t*-test for parametric variables, and the Chi-square test or Fisher's exact test, as appropriate, for categorical variables. Inter-reader agreement was evaluated using intraclass correlation coefficients. Values of $p < 0.05$ were considered to denote significant differences.

Results

Determination of the best cut-off

The best cut-off was calculated in a population of 310 consecutive women. Gender assignment was possible in all 310 fetuses. Sex at birth was available for all newborns except 10. For 10 of the 310 fetuses (3.2%) to which gender was sonographically assigned, information on phenotypic sex at birth was unavailable (lost to follow-up). The mean gestational age at the time of assessment was 12 WG + 3 days (range 11 WG–13 WG + 6 days) and the mean CRL was 63.7 mm (range 46.8–84 mm).

The AGD of the male fetuses was greater than for female fetuses (mean value 6 mm ($IC_{95\%}$ 5.8–6.2) versus 4.2 mm ($IC_{95\%}$ 4–4.3), $p < 0.0001$). The distribution of the fetuses' AGD in male and female is represented in the Fig. 2.

A 4.8 mm cut-off was associated with the best *p* value (minimal *p*-value approach), (Fig. 3).

Optimal threshold accuracy

We constructed a ROC curve in order to confirm the accuracy of the 4.8 mm cut-off (Fig. 4). The score of 4.8 millimeters corresponded to the optimal threshold in terms of clinical utility. Using this cut-off, the sex was correctly determined by ultrasound in 87% of the males (sensitivity) and in 89% of the females (specificity). The chance of being a male when the AGD was more than or equal to 4.8 mm was 91% (positive predictive value) and the chance of being a female when the AGD was less than 4.8 mm was 85% (negative predictive value). Likelyhood ratio (LHR) was 8, area under curve (AUC) 0.93 and $p < 0.0001$ (Table 1).

We divided our population into 3 subpopulations following gestational age: ($\leq$12 GW), (>12 GW –$\leq$13 GW) and (> 13 GW). In the population of women whom gestational age was $\leq$12 WG ($n = 64$), the sex was correctly determined by ultrasound in 66% of the males (sensitivity) and in 100% of the females (specificity). The chance of being a male when the AGD was more than or equal to 4.8 mm was 100% (positive predictive value) and the chance of being a female when the AGD was less than 4.8 mm was 67% (negative predictive value).

In the population of women whom gestational age was >12 GW and $\leq$13 GW ($n = 192$), the sex was correctly determined by ultrasound in 91% of the males (sensitivity) and in 91% of the females (specificity). The chance of being a male when the AGD was more than or equal to 4.8 mm was 92.3% (positive predictive value) and the chance of being a female when the AGD was less than 4.8 mm was 89.8% (negative predictive value).

In the population of women whom gestational age was > 13 GW ($n = 44$), the sex was correctly determined by ultrasound in 100% of the males (sensitivity) and in 64% of the females (specificity). The chance of being a male when the AGD was more than or equal to 4.8 mm was 70% (positive predictive value) and the chance of being a female when the AGD was less than 4.8 mm was 100% (negative predictive value).

These results are reported in Table 1, together with LHRs, AUC and *p*.

Optimal threshold validation (bootstrap correction)

The predictive threshold had an AUC of 0.88 after the 300 repetitions of bootstrap sample corrections. The maximal difference in predicted and observed probabilities of sex was 0.003.

Reproducibility of AGD measurements

Two operators (JSA and JC) separately measured AGD in a 50-woman sample, blinded to each other's measurements. In this