Accuracy of foetal sex determination in the first trimester of pregnancy using 3D virtual reality ultrasound

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Abstract

Purpose: Early detection of foetal sex is becoming more popular. The aim of this study was to evaluate the accuracy of foetal sex determination in the first trimester, using 3D virtual reality.

Methods: 3D ultrasound volumes were obtained in 112 pregnancies between 9 and 13 weeks gestational age, offline projected as a hologram in the BARCO I-Space and subsequently the genital tubercle angle was measured. Separately, the 3D aspect of the genitalia was examined for having a male or female appearance.

Results: Although a significant difference in genital tubercle angles was found between male and female foetuses it did not result in a reliable prediction of foetal gender. Correct sex prediction based on first trimester genital appearance was at best 56%.

Conclusion: Our results indicate that accurate determination of the foetal sex in the first trimester of pregnancy is not possible, even by using an advanced 3D ultrasound technique.

Introduction

Prenatal detection of the foetal sex can be important for medical reasons, such as inherited diseases, e.g., in case of X-linked diseases like Duchenne muscular dystrophy.

Ultrasonographic foetal sex determination is usually performed during the Foetal Anomaly Scan at 18 - 22 weeks gestational age (GA). The reported accuracy ranges between 92 and 100%.[1] With help of the "sagittal sign" in the midline sagittal plane, foetal sex determination is accurate in almost 100% of cases with normal external genitalia after 13 weeks of pregnancy. The differentiation is based on the identification of a "dome sign", representing foetal penis and scrotum, or two or four parallel lines representing labia majora and minora.[2] Not all ultrasonographers are aware of the pitfall that during routine ultrasound before 13 weeks of pregnancy a pronounced genital tubercle of a female foetus may resemble a penis. In the first trimester of pregnancy, the external genitalia of both male and female foetuses appear the same: two labio-scrotal folds with a genital tubercle in the upper midline. In the presence of testes these structures are transformed into a male scrotum and penis respectively. Without the induction by the testes, these structures become the default female labia majora and clitoris.[2]

The angle between the genital tubercle and the body axis has been suggested as a useful in vivo ultrasound parameter for foetal sex determination between 11 and 14 weeks of pregnancy.[3] Male foetuses display a cranially directed tubercle whereas in female foetuses the tubercle is pointed caudally ('sagittal sign'). However, most research in the first trimester has been performed by means of two-dimensional ultrasound[4-7] and at a relatively late GA of more than 11 weeks.[3, 8] Moreover, concerns have been raised about the possibility of determining the sex at an early stage. It has been observed that the genital tubercle angle can change during ultrasonographic examination, caused by an erectile displacement, already at 11 weeks GA.[9]

Recent developments in three-dimensional (3D) ultrasonographic imaging techniques have resulted in improved visualisation of the developing foetus.

A new visualisation approach, based on a 3D virtual reality (3D VR) environment, allows depth perception and interaction with the volume-rendered (ultrasound) data in a more natural and intuitive way compared to 3D images displayed on a 2D screen. This 3D VR approach improves an early detection of surface abnormalities and has already successfully been used in the determination of ambiguous genitalia in a later stage of pregnancy.[10, 11]

Although the foetal genitalia have not been developed entirely, we wanted to investigate whether foetal sex could ultrasonographically be predicted before 13 weeks of pregnancy using a very advanced ultrasound technique. The aim of this study was to evaluate accuracy of foetal sex determination between 9 and 12 weeks of pregnancy, using not only the genital tubercle angle measurement but also a qualitative assessment of the genitalia by means of 3D ultrasound. A secondary aim was to determine the reproducibility of the genital tubercle angle measurement in this early period of pregnancy.

Materials and Methods

Study population

This study comprises women participating in a study on first trimester longitudinal 3D ultrasound measurements conducted at a university hospital to evaluate foetal growth and development using new imaging techniques. Pregnant women who participated were enrolled via the outpatient clinic of the department of Obstetrics and Gynaecology and local midwifery practices. All women received weekly 3D ultrasound scans between 6+0 and 12+6 weeks GA. Only women less than eight weeks pregnant with a singleton pregnancy entered the study for further analysis.

In 2009 we enrolled 141 women from whom at least one volume was obtained in our study. Two pregnancies complicated with trisomy 21 and three with congenital anomalies were excluded. Three multiple pregnancies, three drop outs, 16 miscarriages and two cases with an intrauterine foetal demise had to be excluded as well, leaving 112 inclusions for analysis (**Figure 1**).

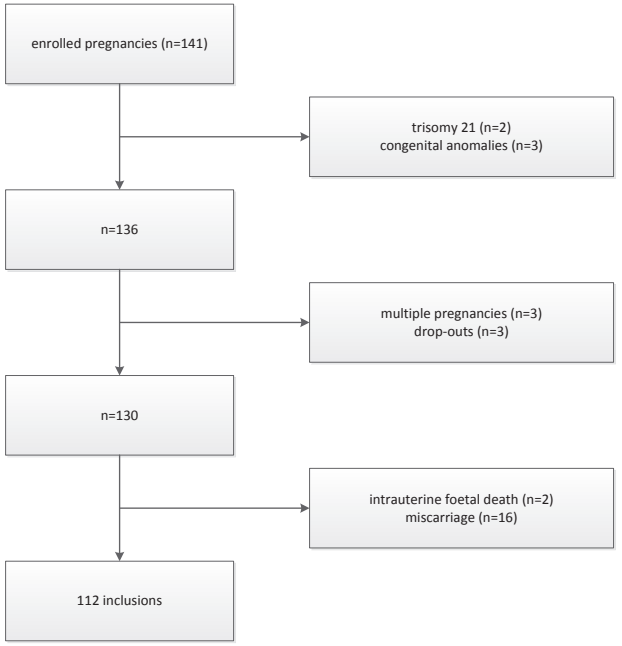

Figure 1: flowchart illustrating inclusions and exclusions of the study population

Ethical approval

All participants signed a written informed consent form and the local medical and ethical review committee approved the study protocol (METC 232.394/2003/177, METC 323.395/2003/178, MEC 2004–227).

Pregnancy dating

The GA was calculated according to the first day of the last menstrual period (LMP) in case of a regular menstrual cycle of 28 days and adjusted for a longer or shorter cycle.[12] In case of a discrepancy in GA of more than seven days between crown rump length (CRL) and the last menstrual period (LMP), or an unknown LMP, the GA was calculated by using CRL at the end of the first trimester. In case of assisted reproductive technology, GA

was determined by the date of oocyte retrieval plus 14 days in pregnancies conceived via in vitro fertilisation with or without intracytoplasmic sperm injection (IVF/ICSI) procedures, from the LMP or insemination date plus 14 days in pregnancies conceived through intrauterine insemination, and from the day of embryo transfer plus 17 or 18 days in pregnancies originating from the transfer of cryopreserved embryos, depending on the number of days between oocyte retrieval and cryopreservation of the embryo.

Material

The ultrasonographic volumes were acquired using a Voluson E8 ultrasound machine (GE Medical Systems, Zipf, Austria) and obtained with a transvaginal scan (GE-probe RIC-6-12-D [4.5–11.9 MHz]). With regard to the safety aspects of first trimester ultrasound the thermal index (TI) and mechanical index (MI) were kept below 1.0, the examiners were qualified and experienced, and the as-low-as-reasonable-practicable (ALARP) principle was respected: the duration of the examination did not exceed 30 minutes, and 3D images were stored for offline evaluation in order to reduce the exposure to ultrasound as much as possible.[13] The 3D volumes were converted to a Cartesian format and visualised in the BARCO I-Space (Barco N.V., Kortrijk, Belgium). This is a four-walled CAVE™-like (Cave Automatic Virtual Environment) virtual reality system. Observers are immersed in a virtual reality room, surrounded by computer-generated passive stereo images, which are projected by eight projectors onto three walls and the floor of a small room. The V-Scope volume-rendering application is used to create a 'hologram' of the ultrasound volume that is being investigated, floating in space in front of the observer. The hologram is viewed through polarising glasses, similar to those used to view 3D movies, to create the perception of depth. The hologram can be manipulated by means of a virtual pointer, controlled by a wireless joystick. This joystick also operates a measuring tool to trace lines and measure angles and volumes. In addition tracking of the viewer's head allows the computer to provide the correct perspective and motion parallax, which in combination with the stereoscopic images, helps in discerning fine details and in the understanding of 3D structures in the volumes. For our study the 3D volumes were resized (enlarged), rotated and cropped when necessary and grey-scale and opacity values were adjusted for optimal image quality.[14, 15]

Measurements

In the BARCO I-Space the 3D foetal hologram was placed in a horizontal position and callipers were placed in a mid-sagittal cross section for a very precise CRL measurement. A second line was positioned in the central axis of the genital tubercle and the angle between the genital tubercle and the CRL line, being the central bodyline, was measured[3] (**Figure 2**). Because of a more precise positioning of the callipers we measured the angle between the tubercle and the upper part of the CRL line. The genital tubercle angle was calculated afterwards by subtracting the measured angle from 180 degrees.

Apart from this quantitative prediction, two experienced ultrasonographers (MHE and NE) examined the foetal genitalia for a male or female appearance separately without knowing their results or the duration of pregnancy. All volumes in which an angle measurement had been performed were projected in the BARCO I-Space and were scored as male, female or unknown. All examiners were blinded for the outcome of pregnancy. After all measurements had been performed the results were compared to the neonatal sex at birth as reference.

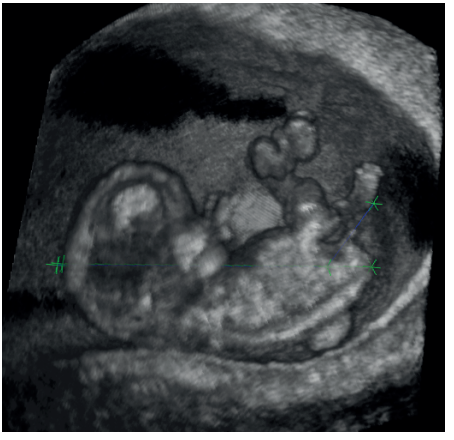

Figure 2: measurement of the genital tubercle angle of a male foetus at a GA of 10 weeks and 2 days in the BARCO I-Space

Statistical analysis

The measured angle by GA was described with ordinary least squares regression analysis for male and female foetuses separately. The dependent variable was measured angle; the independent variables were GA and square of GA.

Predictive accuracy of foetal sex was evaluated with binary logistic regression, with foetal sex as dependent variable and GA, square of GA, measured angle and square of measured angle as independent variables (backward conditional analysis). Predictive value was assessed as the proportion of correct predictions. A $p<0.05$ (two-sided) was considered a statistically significant difference.

Reliability of the measurements was depicted with Bland Altman plots indicating the bias (the systematic of mean difference between two measurements) and limits of agreement (LOAs). Intraclass correlation coefficients (ICCs, two way mixed, single measures, absolute agreement) of intraobserver and interobserver angle measurements were added.

Reliability of the qualitative prediction was determined by calculating Cohen's kappa. We used the directives of reproducibility as described by Landis and Koch[16] (poor: $\kappa<0$; slight: $\kappa=0.00–0.20$; fair: $\kappa=0.21– 0.40$; moderate: $\kappa=0.41–0.60$; substantial: $\kappa=0.61–0.80$; almost perfect $\kappa>0.80$).

Reproducibility

All measurements were performed offline. Intrarater agreement was assessed by single repeated measurement of 30 randomly selected volumes (16 male, 14 female) by the same examiner (HB). Interrater agreement was evaluated by comparing the measurements of the same 30 volumes performed by another examiner (MR).

Results

From the 112 pregnancies 426 volumes were obtained for evaluation from 9 until 13 weeks GA. Median maternal age was 32.9 years (range: 18.9-42.7). 62.5% were nulliparous and 25.9% had a history of two or more miscarriages. 70.5% conceived naturally. Median birth weight was 3390 grams (range: 450-4700) and median GA at delivery was 39 weeks + 4 days (range: 26+4 - 42+0) (**Table 1**). No anomalies of the genitalia were observed postpartum.

In 102 out of 112 singleton pregnancies at least one measurement was feasible, concerning 53 male (range 9+0 - 12+6 weeks+days GA) and 59

Characteristic	**Median (range) or percentage**
Mothers (n=112)	
Maternal age (years)	32.9 (18.9-42.7)
Gravidity	2 (1-10)
Parity	
0	62.5%
1	27.7%
≥ 2	9.8%
Miscarriages ≥ 2	25.9%
Conception mode	
Natural	70.5%
IVF or IVF/ICSI	27.7%
Intrauterine insemination	1.8%
Gestational diabetes	5.4%
Hypertensive disorders	8.9%
Foetal growth retardation	3.6%
Newborns (n=112)	
Female	52.7%
Birth weight (grams)	3390 (450-4700)
GA at delivery (weeks)	39+4 (26+4 to 42+0)

Table 1: general characteristics of the study population
IVF: in vitro fertilisation, ICSI: intracytoplasmic sperm injection, GA: gestational age

female (range 9+0 - 12+6 weeks+days GA) foetuses. A genital tubercle angle measurement could be measured in 235 of 426 3D volumes (total 55.2%; male: 116/206=56.3%, female: 119/220=54.1%). The feasibility per pregnancy week is described in **Table 2**. The median number of angle measurements was 2 (range: 1-4) for male as well as female foetuses. **Table 3** displays the regression lines corresponding to the pattern seen in male and female foetuses.

Figure 3 points to a considerable overlap between the measurements of male and female foetuses. The curves follow more or less the same trend between 60 and 80 days GA, but from then (approximately 11 weeks GA) onwards a different pattern is seen: the mean angle of male foetuses increases

Week	Male	%	Female	%	Total	%
9	26/54	48.1	25/54	46.3	51/108	47.2
10	31/54	57.4	41/55	74.5	72/109	66.1
11	36/50	72.0	37/58	63.8	73/108	67.6
12	23/48	47.9	16/53	30.2	39/101	38.6
Total	116/206	56.3	119/220	54.1	235/426	55.2

Table 2: success percentages of genital tubercle angle measurements for male- and female foetuses (number of feasible measurements / number of available volumes; %) by gestational age (expressed in complete weeks)

	Male Beta-coefficient (95%CI of beta-coefficient)	P	Female Beta-coefficient (95%CI of beta-coefficient)	P
Constant	627.13 (40.98 ; 1213.27)	0.04	189.73 (-357.24 ; 736.70)	0.49
Gestational age (days)	-14.24 (-30.22 ; 1.74)	0.08	-1.48 (—16.42 ; 13.45)	0.84
Square of gestational age	0.09 (-0.02 ; 0.20)	0.11	-0.00 (-0.11 ; 0.10)	0.94
Adjusted R^2	0.23		0.41	
P of regression		<0.001		<0.001

Table 3: relationship between measured angle and gestational age for male and female foetuses separately
CI: confidence interval

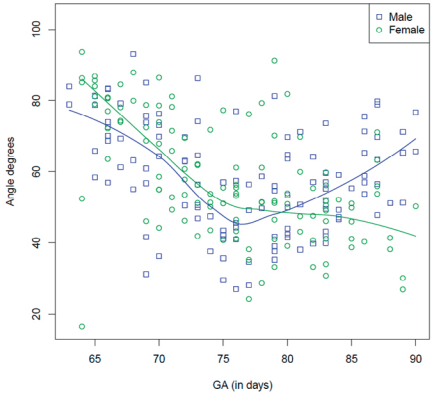

Figure 3: scatterplot of measured genital tubercle angles (circles: female foetuses, squares: male foetuses) against gestational age; the curves represent the fitted regression curves (green line: female foetuses, blue line: male foetuses)

whereas the mean angle of female foetuses slightly diminishes. GA, square of GA, measured angle and square of measured angle were not significantly associated with a correct prediction of foetal sex. The power to predict sex was moderate, only 54.9% of measurements were correctly predicted (**Table 4**). The receiver operating characteristic (ROC) curve is shown in **Figure 4**.

Table 5 displays the results of the qualitative prediction, based on the genital appearance, per gestational week. Correct prediction was at best 56.4% (range: 28.8%-56.4%). **Figure 5** illustrates a pronounced genital tubercle in a female foetus. In 6.9%-23.0% of the cases foetal sex prediction was not possible, although angle measurements in these cases were feasible.

With regard to the angle measurements the Bland and Altman plots are shown in **Figures 6** (intraoperator agreement) and **7** (interoperator agreement). Reproducibility was excellent. The intraclass correlation coefficients of intra- and interoperator angle measurements were 0.97 (95% confidence interval [CI]: 0.94 - 0.99) and 0.95 (95% CI: 0.90-0.98), respectively.

The measure of agreement of the qualitative sex prediction was fair: κ = 0.24 (95% CI: 0.13-0.34) with the prediction 'unknown' taken into account.

	OR	95%CI of OR	P
Constant	$2.35 \cdot 10^{-4}$		0.78
Gestational age (days)	1.37	0.27 ; 7.29	0.70
Square of gestational age	1.00	1.00 ; 1.01	0.71
Angle (degrees)	0.87	0.73 ; 1.02	0.11
Square of angle (degrees)	1.00	1.00 ; 1.00	0.09
Proportion correct predictions	54.9%	45.2 ; 64.6	
Area under the curve	0.61	0.50 ; 0.72	

Table 4. Logistic regression analysis, backward conditional analysis.
OR: odds ratio. CI: confidence interval.

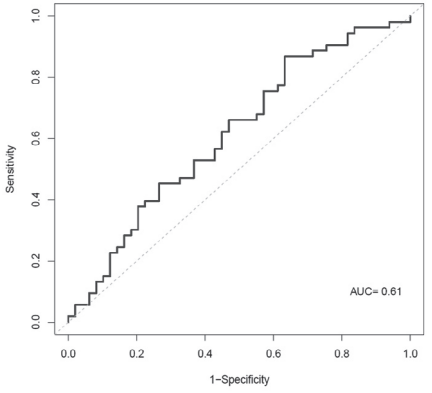

Figure 4: receiver operating characteristic (ROC) curve of foetal sex determination by measuring the genital tubercle angle

Discussion

Although the relation between genital tubercle angle measurement and GA differs significantly between male and female foetuses, our results indicate that prediction of the foetal sex by measuring the angle of the genital tubercle in the first trimester of pregnancy before 12 weeks GA in individual cases was not reliable. Furthermore, prediction of the foetal sex based on genital appearance in this period was at best 56.4%.

GA (weeks)	Rater 1			Rater 2			Total
	True	False	Unknown	True	False	Unknown	
9	15 (28.8%)	31 (59.6%)	6 (11.5%)	22 (42.3%)	22 (42.3%)	8 (15.4%)	52
10	38 (52.8%)	29 (40.3%)	5 (6.9%)	32 (44.4%)	32 (44.4%)	8 (11.1%)	72
11	35 (47.3%)	28 (37.8%)	11 (14.9%)	31 (41.9%)	26 (35.1%)	17 (23.0%)	74
12	17 (43.6%)	16 (41.0%)	6 (15.4%)	22 (56.4%)	10 (25.6%)	7 (17.9%)	39

Table 5: the results of foetal sex prediction by determining a male or female appearance of the genitalia in the Barco I-Space
True: correct prediction of foetal sex. False: wrong prediction of foetal sex. Unknown: prediction was not possible.

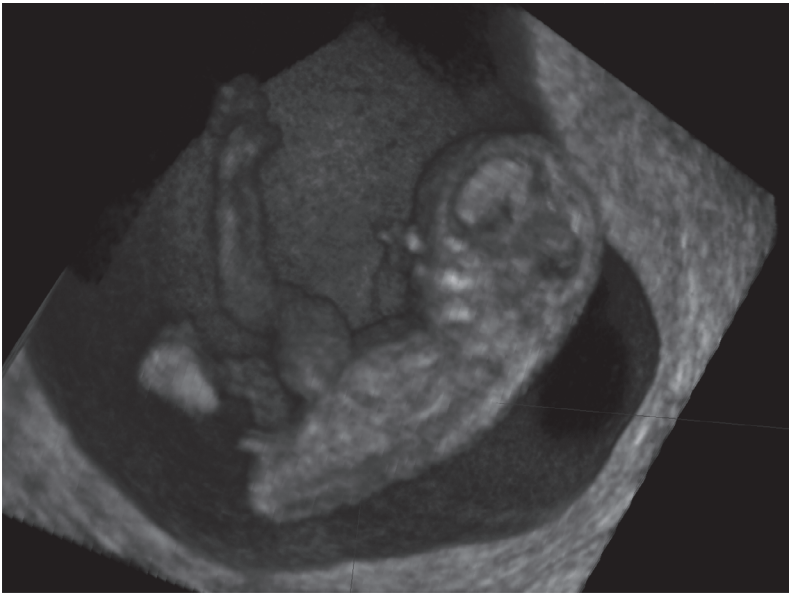

Figure 5: a pronounced genital tubercle in a female foetus at a GA of 10 weeks and 2 days in the BARCO I-Space

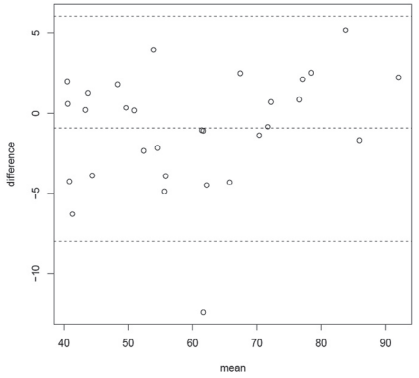

Figure 6: Bland and Altman plot for intrarater agreement
Data shown are the differences (second minus first) of measured genital tubercle angles of one rater plotted against the mean of the two measurements. The dashed outer lines show the 95% limits of agreement (LOAs), the dashed middle line represents the mean difference

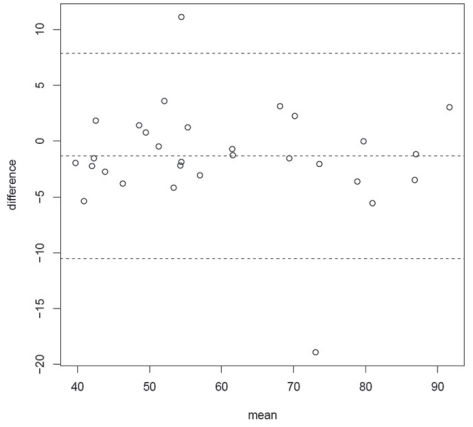

Figure 7: Bland and Altman plot for interrater agreement
Data shown are interrater differences of measured genital tubercle angles plotted against the mean of the two rater measurements. The dashed outer lines show the 95% limits of agreement (LOAs), the dashed middle line represents the mean difference.

Most research on foetal sex prediction in the first trimester has been performed at the end of the first trimester. To the best of our knowledge, we are the first who investigated the possibilities of angle measurement and sex prediction before 11 weeks of pregnancy.

Trying to push the borders we employed the most advanced technique, namely the 3D VR approach. 3D VR is superior in very precise and accurate positioning of lines and it has already shown its benefits in examining surface abnormalities and the foetal genitalia.[10, 11] Although this system is not widely available, a desktop VR system has been developed with comparable 3D views, using polarising glasses and an optical tracking system, implementing the same 3D VR measurement tools. We used the CRL line instead of a line tangentially to most dorsal part of the body since the former can be drawn more precisely.

Until 11 weeks GA we could not find a difference in angles between male and female foetuses. From 11 weeks onwards, mean male angles start to increase whereas female angles still decrease, which might be caused by foetal erectile movements.[9] Hsiao et al. and Youssef et al. investigated the foetal sex from 11+0 until 13+6 weeks GA and found the success rate of correctly predicting the sex to increase with advancing GA.[3, 4] Efrat et al. investigated foetal sex prediction in 656 women and predicted this incorrectly in 8.5% of eventually female foetuses until 12+4 GA.[5] From 13+0 GA onwards, a success rate of 100% was achieved. Lower success rates were reported by Chelli et al: in 85.7% foetal sex prediction was accurate, yet in male foetuses an increase in the genital angle was observed with increasing CRL.[6] Also Michailidis et al. observed a lower correct prediction rate of the foetal sex in offline studying 3D volumes by two different examiners of 85.3%.[8] Mazza et al. concluded in their study of 2374 foetuses not to assign foetal sex below a biparietal diameter (BPD) of 22 mm (which equals 12+5 GA[17]). From that moment onwards, however, an accuracy rate of 99% was obtained.[7] Recently Manzanares et al. investigated between 11 and 14 weeks GA foetal sex prediction and factors influencing correct prediction. After excluding 6.5% of the foetuses in which an intermediate angle

(not sufficiently discriminating between sexes) was found, in the remaining foetuses an overall correct prediction rate of 87.5% was achieved. Correct prediction was positively correlated with GA and CRL and negatively with maternal BMI.[18]

In view of the huge overlap even at 13 weeks GA and the inability to predict foetal sex in individual cases, all these findings are in accordance with ours. Until about 13 weeks GA accuracy is low and after 13 weeks GA, the visibility of the difference between male and female angles increases with advancing GA. It is a limitation of our study that we have only data available until 13 weeks GA.

Another limitation is the low feasibility rate of angle measurement of 55.2%. The measurements however, were performed offline on stored data. The low feasibility figure is a result of non-targeted scanning, acoustic shadowing and movement artefacts. If it was possible to measure the angle, until 11 weeks GA foetal sex could not be predicted whereas from 11 to 13 weeks the angle measurement insufficiently discriminated between male and female foetuses. In all of these foetuses, in up to 23.0% it was not possible at all to predict foetal sex by looking at the appearance of the genitalia and, if prediction was possible, in at best half of the cases prediction was correct. This also means a warning for clinicians that a male appearance of a pronounced genital tubercle before 13 weeks GA is not unusual in female foetuses (**Figure 5**).

Also the distance between base of the genital tubercle and the caudal end of the foetus can be used to differentiate between foetal sex. In newborns in males this distance is twice as long as in females. This measurement has recently been described for the first time in pregnancies between 11 and 14 weeks GA but correct prediction differed between 64% and 100% depending on GA.[19]

A different non-invasive way of determining foetal sex is by obtaining cell-free foetal DNA in maternal blood by detecting DNA sequences of the Y

chromosome.[20] This technique has a proven sensitivity and specificity of 100% and it can already be performed in the first trimester of pregnancy, from 7 – 9 weeks GA onwards.[21, 22] This test is, although more cumbersome compared to bedside testing and more expensive, superior to ultrasonographic sex determination in this early stage.

Conclusion

Our results indicate that accurate determination of the foetal sex by genital tubercle angle measurement or looking at a male or female genital appearance in the first trimester of pregnancy is very limited, even by using very high quality 3D Virtual Reality ultrasound techniques. We therefore conclude that if foetal sex has to be determined other techniques like DNA sequencing should be used.

Acknowledgments

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