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Anogenital Distance for Detection of Fetal Sex in First Trimester of Pregnancy

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ABSTRACT

Background: Expectant parents are extremely interested in learning the gender of the fetus during the early stages of pregnancy. Yet, clinicians are also interested in identifying the gender of fetuses at increased risk of inheriting gender-dependent genetic diseases. In the presence of hereditary illnesses associated with gender, it becomes a necessity rather than an interest.

Objective: The aim of the current study is to evaluate the reliability of early-trimester fetal sex diagnosis of pregnancy, as well as the relationship between anogenital distance (AGD) in the first trimester and fetal sex determination.

Patients and methods: A prospective cohort study was conducted at the Department Obstetrics and Gynecology of Zagazig University Hospitals, from August 2021 to December 2022. The study included 245 pregnant women, aged between 18 and 35 years. Participants were singleton pregnancy with gestational age from 11 to 13 weeks (+6 days). The enrolled pregnant women in our study were divided into 3 groups; Group 1 included fetuses that aged 11 weeks (+6 days), Group 2 included fetuses that aged 12 weeks (+6 days), and Group 3 included fetuses that aged 13 weeks (+6 days). **Result**: Our research indicated no statistically significant difference between AGD in groups 2 and 3 (P=0.758), although there was a statistically significant difference between Group 1 and both Groups 2 and 3 (P<0.001), thus, both groups 2 and 3 were combined into one group. It was established that the optimal cut-off value for the merged group was 4.9mm, AUC 0.961 (P<0.000). The sensitivity was 93.41%, specificity was 86.49%, positive predictive value 89.47%, negative predictive value 91.43%, and likelihood ratio 6.91.

Conclusion: This research backs the use of fetal AGD measurement as a new ultrasonographic technique signal for determining a baby's gender early on. This procedure appears to be accurate, especially after 12 weeks of pregnancy. It is recommended measuring AGD during the first trimester of early pregnancy for accurate gender prediction.

Keywords: Fetal sex determination, Anogenital Distance, Pregnancy, Cohort study, Zagazig University.

INTRODUCTION

The gender of the fetus prior to delivery has been of interest to both the physician and the family. When gender-specific genetic diseases are present, it becomes a need rather than a desire to have children [(1)].With X-linked recessive inherited illnesses, only male fetuses are affected, whereas only female fetuses are affected by congenital adrenal hyperplasia. Thus, early diagnosis of fetal gender is essential [(2)].

The chorionic villus biopsy under ultrasonography is the most accurate way for establishing the gender of an embryo, although it is invasive and carries a 0.5% to 1.0% risk of fetal loss [(3)].

Analysis of mother's blood for cell-free fetal DNA is another way for establishing gender. This is believed to be a noninvasive technique, but is pricey and is not available in all regions [(4)].

US of morphological criteria and ultrasound in the second trimester are the easiest non-invasive methods for determining the fetal gender (For men, the penis and scrotum; for women, the major and minor labia). After 20 weeks of pregnancy, this simple and readily available procedure is 100% correct in the absence of gender anomalies [(5,6)].

Owing to biological limitations like small size and insufficient contrast between the genitalia or technical limitations like the difficulty in
accurately determining the midsagittal plane, the accuracy of a fetal gender diagnosis diminishes prior to the second pregnancy trimester [(7)].

Calculating the angle between the genital tubercle and a horizontal line on the lumbosacral skin during the first trimester can help determine the gender in the midsagittal plane of the fetus as a result of advancements in ultrasound resolution [(8,9)]. This treatment has a sensitivity of 100% for gender determination after 13 weeks of pregnancy; however, its sensitivity between 11 and 12 weeks is modest [(10)].

The aim of the current study is to evaluate the reliability of early-trimester fetal sex diagnosis of pregnancy, as well as the relationship between anogenital distance (AGD) in the first trimester and fetal sex determination.

PATIENT AND METHODS

A prospective cohort study was conducted at the Department Obstetrics and Gynecology of Zagazig University Hospitals, from August 2021 to December 2022.

The study included 245 pregnant women, aged between 18 and 35 years. Participants were singleton pregnancy with gestational age from 11 to 13 weeks (+6 days). Patients who refused to provide consent or with multifetal gestation were excluded.

The enrolled pregnant women in our study were divided into 3 groups;

Group 1 included fetuses that aged 11 weeks (+6 days). A total of 80 fetuses were studied in this group, out of which 39 were boys and 41 were girls. The mean age for boys was 11 weeks (+4 days) with SD 2 days

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and for girls was 11 weeks (+4 days) with SD 1 days (P=0.420). While mean AGD for boys was 5.19 (SD 0.3) and for girls was 4.02 (SD 0.53) (P<0.001).

Group 2 included fetuses that aged 12 weeks (+6 days). A total of 80 fetuses were studied in this group, out of which 41 were boys and 39 were girls.

The mean age for boys was 12 weeks (+3 days) with SD 1 day and for girls was 12 weeks (+3 days) with SD 2 days (P=0.148). While mean AGD for boys was 5.84 (SD 0.44) and for girls was 4.21 (SD 0.24) (P<0.001).

Group 3 included fetuses that aged 13 weeks (+6 days). A total of 85 fetuses were studied in this group, out of which 52 were boys and 33 were girls. The mean age for boys was 13 weeks (+2 days) with SD 2 days and for girls was 13 weeks (+3 days) with SD 2 days (P=0.167). While mean AGD for boys was 5.30 (SD 0.31) and for girls was 4.74 (SD 0.21) (P<0.001).

All moms submitted to ultrasound by the same ultrasound team. It evaluated the fetal AGD, while it was neutral (neither hyperextensive nor hyperflexive) mid-sagittal plane. By measuring the length from the crown to the rump, the snapshot was utilized to determine the exact gestational age (CRL).

The inferior genital appendage end of AGD was calculated starting from the caudal end of the fetus (**Figure 1**). AGD in each group was cut out using the receiver operating characteristics (ROC) curves. The gender of the infant was recorded for each participant. Follow up until second trimester, after delivery to ensure fetal gender determination.

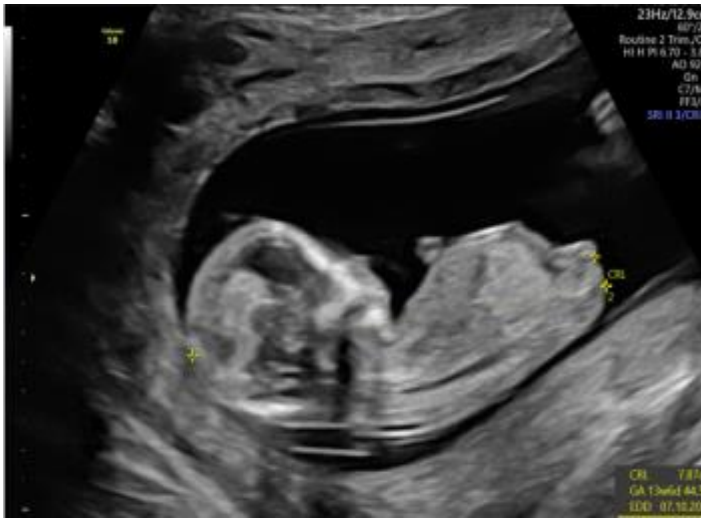

Figure (1): Measurement of CRL and AGD.

Ethical Consideration:

This study was ethically approved by the Institutional Review Board [IRB] of the Faculty of Medicine, Zagazig University (#ZUIRB 6960-23-5-2021). Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS IBM Corporation, version 20.0, Armonk, NY). Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD). One-way analysis of variance (ANOVA) was utilized to determine the disparity between groups. If statistically significant differences (P<0.05) were seen, Tukey's post hoc comparison was used to compare each group to each other.

The diagnostic performance is measured using ROC curves of AGD in predicting fetal gender was examined. To optimize the sum of sensitivity and specificity, the ideal cutoff values were chosen. Also, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Likelihood Ratio (LR) were estimated. P value ≤0.05 was considered to be statistically significant.

RESULTS

Diagnostic performance of AGD for detecting gender: To counteract the impact of age on AGD, the optimal cutoff for determining gender by AGD was established separately in each group. **Group 1:** ROC curve analysis revealed that the ideal cut-off value for predicting gender in group 1 was 4.5 mm. By using this cut-off, ultrasonography was able to determine the fetal gender with accuracy in 100 % of male fetuses (sensitivity) and in 82.9 % of female fetuses (specificity). The possibility to be AGD more than or equal to 4.5 mm increased the likelihood of being a male by 84.87% (PPV), while AGD less than 4.5 mm increased the likelihood of being a female than 4.5 mm was 100% (NPV) **(Table 1).**

Table (1): Characteristics and sensitivity in Group 1.

Cut-off	AUC	P-value	Sensitivity	Specificity	LR	PPV	NPV
4.5	0.967	0.000	100%	82.9%	5.86	84.87%	100%

Figure 2 shows a case of 33 years old pregnant woman [G3P2, Gestational age 11weeks (+ 6 days) by CRL; AGD is 4.1.

Figure (2): Follow up showed that the gender is female.

Group 2: The optimal cut-off value for predicting gender in group 2 was 4.5 mm, the area under the curve (AUC) was 0.988 (P<0.000). Using this cut-off, fetal gender was correctly established by ultrasound in 100% of male fetuses and in 84.6 % of female fetuses. The possibility to be a male when the AGD was ≥4.5 mm was 87.23% and the possibility to be a female when the AGD was <4.5 mm was 100%. LR was 6.5 **(Figure 3).**

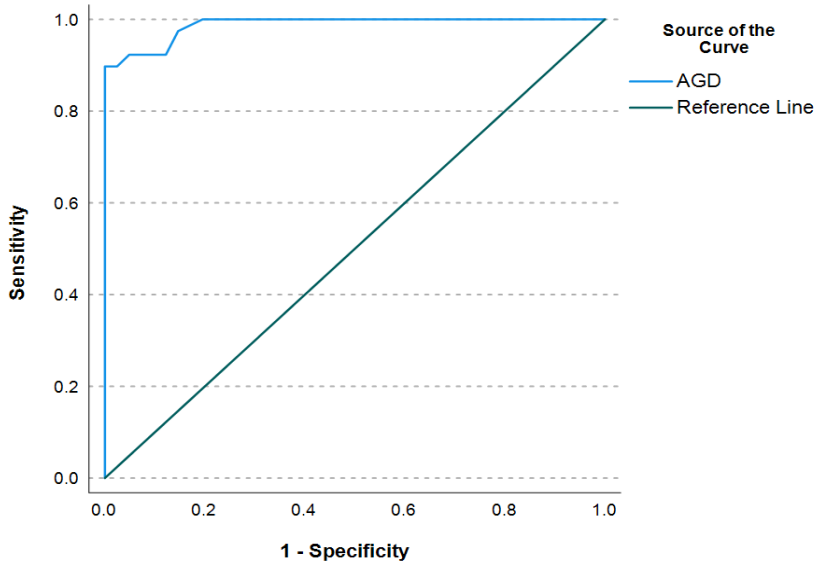

Figure (3): ROC curve to determine gender of fetuses in first trimester US scan (12 weeks +6 days).

Group 3: The optimal cut-off value for predicting gender in group 3 was 4.9 mm, the area under the curve AUC was 0.928 (P<0.000). Using this cut-off, fetal gender was correctly established by ultrasound in 94.23% of male fetuses and in 81.82 % of female fetuses. The possibility to be a male when the AGD was ≥4.9 mm was 89.09 and the possibility to be a female when the AGD was <4.9 mm was 90%. LR was 5.18 **(Figure 4).**

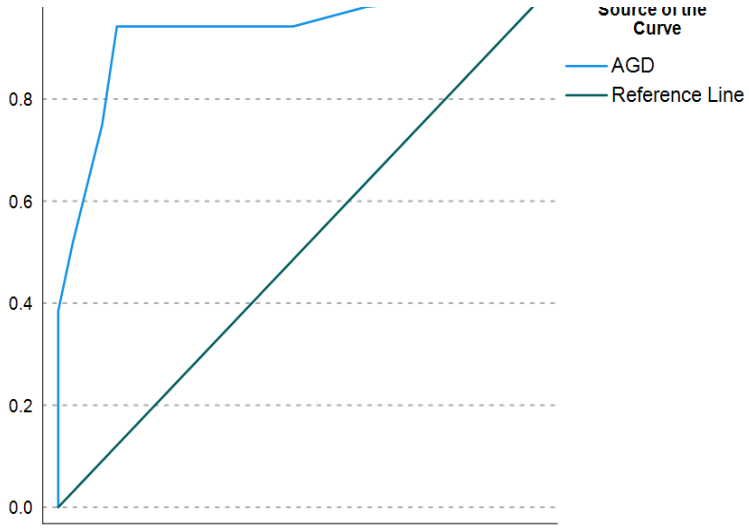

Figure (4): ROC curve to determine gender of fetuses in US scan (13 weeks +6 days).

As there was no significant difference between AGD in group 2 and 3 (P=0.758) while there was a significant difference between group 1 and both group 2 and 3 (P<0.001). So both groups 2 and 3 were combined into one group. The best cut-off value for the combined group was determined to be 4.9 mm, AUC 0.961 (P<0.000). The sensitivity was 93.41%, specificity was 86.49%, PPV 89.47%, NPV 91.43%, and LR 6.91 **(Figure 5).**

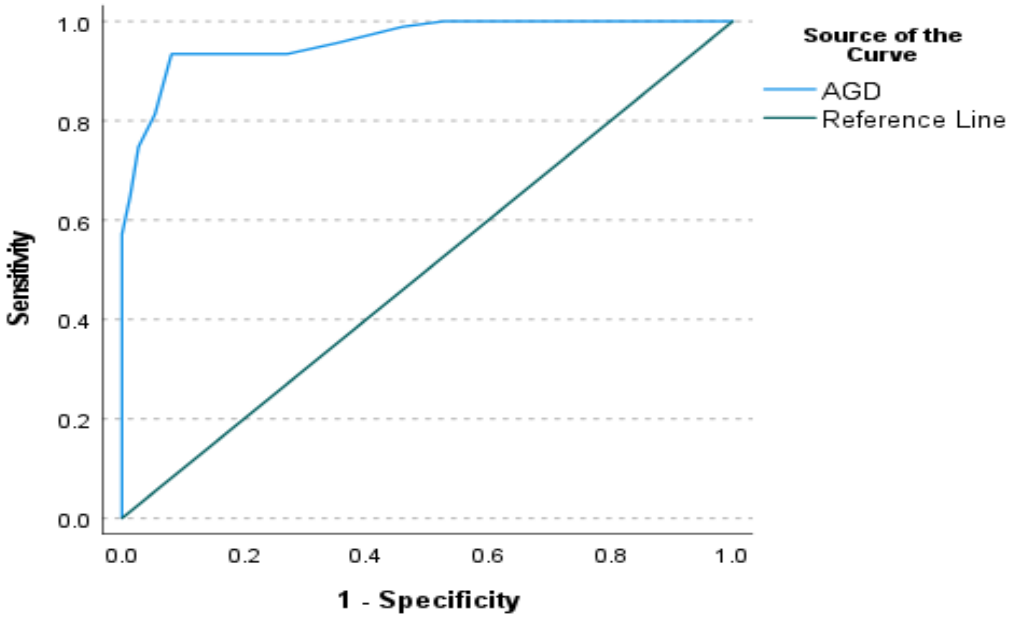

Figure (5): ROC curve to determine gender of fetuses in US scan (12w +6 days).

Table 2 showed that there was no difference between gender in second trimester and post-delivery**.**

Table (2): Gender of all cases in each group is detected at second trimester and ensured after delivery.

Variable	Group 1		Group 2		Group 3	
	Male N.39	Female N.41	Male N.41	Female N.39	Male N.52	Female N.33
Gender in second trimester	48.75%	51.25%	51.25	48.75	61.18	38.82
Gender after delivery	48.75%	51.25%	51.25	48.75	61.18	38.82

DISCUSSION

As regards diagnostic performance of AGD for detecting gender in group 1, the optimum cut-off value for predicting gender in group 1 was 4.5 mm, and AUC was 0.967 (P<0.000). Ultrasound properly identified the gender of 100% of male fetuses (sensitivity) and 82.9% of female fetuses (specificity).

As regards diagnostic performance of AGD for detecting gender in group 2, the optimal cutoff value for predicting gender in group 2 based on the results showed that ROC curve analysis was 4.5 mm. Ultrasound properly determined the gender of 84.6% of female fetuses and 100% of male newborns. When the AGD was ≥4.5 mm, the probability of being male was 87.23%, whereas the probability of being female was 100%.

As regards diagnostic performance of AGD for detecting gender in group 3, the most effective cut-off value was discovered by analysis of ROC curves for predicting gender in group 3 was 4.9 mm, with AUC of 0.928 (P<0.000). Ultrasound accurately determined the gender of 94.23% of male fetuses and 81.82% of female fetuses.

Ultimately, our study results revealed showed there was no significant difference in AGD although Groups 2 and 3 differed significantly from group 1 and both groups (P=0.758) 2 and 3 (P<0.001).

Hence, groups 2 and 3 were merged into a single group. The best cut-off value for the combined group was determined to be 4.9mm, AUC 0.961 (P<0.000). The sensitivity was 93.41%, specificity was 86.49%, PPV 89.47%, NPV 91.43%, and LR 6.91.

Gender of all cases of each group is detected at second trimester and ensured after delivery Group 1 (males 39) (females 41), Group 2(males 41) (females 39), Group 3 (males 52) (females 33).

To the best of our knowledge, there is a paucity of studies in literature assessing the fetus sex determination via anogenital examination during the first trimester of pregnancy distance, and that represents a strength point of our study.

These findings are consistent with earlier research. **Alfuraih *et al.*** [11] A retrospective cohort study a study of 313 singleton pregnancies between 11 and 13 plus 6 gestational weeks that examined the accuracy of sonographically measured AGD in determining the gender of the fetus during the first trimester and established normal reference centiles for AGD found that male fetuses measures that were longer than those of female fetuses by 14.8% (P<0.001). Each gestational week's mean AGD measurements varied significantly as well, from 10.7% in week 13 to 17.4% in week 11, and AGD was closely linked to gestational age (r=0.573, P<0.001).

The receiver operator characteristic study evaluated a number of cutoffs, and the best at a total cutoff value of 6.00 mm, the highest Youden index was obtained, which was 29, with sensitivity, specificity, and area under the curve values of 69%, 60%, and 0.686, respectively. This shows that there is no discrimination [11].

Also, **Najdi *et al.*** [2] An reliable ultrasonography ultrasound measurement of the fetus's AGD in the first trimester was used to predict the gender of the fetus in a cross-sectional study involving 316 expectant mothers carrying singletons between 11 and 13 weeks (SD 6) days were split depending to the age of the pregnancy, into three groups, much like in our study.

Najdi *et al.* [2] 87 fetuses in all were assessed in group (1), 60 of which Depending on the gestational age, there are three groupings of 4.01 (SD 0.81) mm and 27 fetuses with an average AGD of, were male 4.68 (SD 0.74) mm (P=0.0019). Group 2 consisted of fetuses aged 12 weeks to 12 weeks and six days. There were a total of 180 fetuses examined. About 102 of these fetuses were female, with a mean gender of female AGD was 4.25 0.57 mm, and 78 of the subjects were male of 4.67 (SD 0.59) mm (P<0.00001). Group 3 included 48 fetuses aged between 13 weeks to 13 weeks, 6 days of age. Of these, 23 fetuses were female and 27 were male. The average AGD of the females was 4.65 (SD 0.71) mm and of the boys was 6.06 (SD 0.85) mm (P<0.0001).

The best cutoff for identifying gender was determined for each group separately in order to take age out of the equation for AGD. The regions every three weeks, under the curve were compared. There were no obvious differences between the groups 2 and 3 (P=0.10), whereas a substantial difference was seen between groups 2 and 3, but not between group 1 and groups 2 and 3 (P=0.0003); Hence, the samples from groups 2 and 3 were merged into a single group. The optimal cutoff was determined to be 4.9 millimeters. Calculations were done for both the specificity and sensitivity, as 95% and 89.6% [2].

Conclusively, **Najdi *et al.*** [2] Researchers found that the AGD was significantly higher in male embryos than in female embryos. Pregnancies older than 12 weeks with a threshold of 4.9 mm had the best results. Based on this, 88 percent of male embryos and 95 percent of female embryos had their gender correctly determined.

Arfi *et al.* [12] to determine the gender of fetuses during the first trimester of pregnancy by evaluating AGD with gestational ages between 11 and 13 weeks and 6 days, we enlisted 310 women with singleton pregnancies in a prospective study. The researchers attempted to develop a limit for precise fetal gender determination by assessing AGD. This threshold was

determined to be 4.8 mm using the ROC curve. AGD's capacity to determine whether an embryo will be male of ≥4.8 mm was 92.3% and at AGD ≤4.8 mm, the chance of predicting a female embryo was 89.8%.

Later, **Sipahi *et al.*** [(1)] 111 women who were carrying singletons between 11 and 13 weeks and 6 days were involved in a prospective cross-sectional study to establish the fetal gender accurately in the first trimester using AGD that was divided into three groups based on the number of weeks of gestation (≤12 gestational weeks n=24, >12 gestational weeks – ≤13 gestational weeks n=65, >13 gestational weeks n=22). Gender was found with an accuracy of 92.3% for females and 63.6% for males at ≤12 WG. When an AGD 4.8 mm was found by ultrasound, the odds of a girl was 75%, whereas the likelihood of a male was 87.5%.

Gender was accurately determined in all cases for female expectant moms with a gestational age between >12 gestational weeks and 13 gestational weeks, and in 73.9% of cases for male expectant mothers. the likelihood of being a woman was 87.5% when an AGD <4.8 mm When an AGD 4.8mm was seen using ultrasound, there was a 100% chance that it was a man.

Gender was identified with 92.3% accuracy for females and 100% accuracy for males among the population of mothers whose gestational age was greater than 13 weeks. The likelihood of being a woman during an AGD was 100%. When an AGD was found by ultrasound at 4.8 mm, the likelihood of it being a boy was 90%.

The optimal cut-off point for AGD to predict fetal gender, as shown by ROC curve analysis, is 4.8 mm with 76.7 and 95.6%, respectively, for sensitivity and specificity, between 11 and 13 weeks [(1)].

This study's strengths include its prospective design, its setting at a single tertiary care hospital, and the fact that no patients were lost to follow-up during the study period. It presented the use of AGD to determine fetal sex as a new way for diagnosing fetal sex, particularly in the first trimester.

Notable study drawbacks include a comparatively smaller sample size compared to previous studies and the lack of a multicentric design, which poses a high risk of publication bias. Another drawback is the absence of association with CRL for fetal gender prediction, which may underestimate the excellent predictive accuracy of AGD for fetal gender prediction. Further extensive researches are required to confirm the results.

CONCLUSION

This research backs the use of fetal AGD measurement as a new ultrasonographic technique signal for determining a baby's gender early on. This procedure appears to be accurate, especially after 12 weeks of pregnancy. It is recommended measuring AGD during the first trimester of early pregnancy for accurate gender prediction.

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Conflicts of interest: There are no conflicts of interest, according to the authors.

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