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# Anogenital Distance for Detection of Fetal Sex in First Trimester

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## ABSTRACT

**Background:** The determination of fetal sex in the first trimester has significant implications, not only for familial curiosity but more critically in sex-linked genetic disorders. Traditional methods range from invasive procedures with associated risks to non-invasive techniques that may lack universal accessibility or come with high costs. Ultrasonography in the second trimester, though effective, offers a delayed resolution that may not be ideal for early intervention in cases of sex-linked diseases. **Objective:** This study aimed to assess the utility of anogenital distance (AGD) measurements in the first trimester as a reliable, non-invasive predictor of fetal sex, thereby facilitating earlier diagnosis and management of sex-linked conditions. **Methods:** This prospective cohort study was conducted at Benha University Hospitals' Feto-maternal Unit, 245 pregnant women within 11-13 weeks +6 days of gestation were included. The AGD was measured via ultrasound, avoiding positional bias, to predict fetal sex.

**Results:** The study delineated a clear distinction in AGD measurements between male and female fetuses, with males presenting significantly longer AGD. Optimal AGD cut-offs were established at 4.5 mm for weeks 11 to 12+6 days with an AUC of 0.967-0.988, indicating high diagnostic accuracy. At 13 weeks to 13 weeks +6 days, a cut-off of 4.9 mm was identified, with an (Area under the Curve) AUC of 0.928. The predictive accuracy of fetal sex using AGD was substantiated by a 100% confirmation rate of sex determination in the second trimester and post-delivery.

**Conclusions:** AGD measurement in the first trimester presents a highly accurate, non-invasive method for early fetal sex determination.

**Keywords:** Anogenital distance; Fetal sex determination; First trimester; Ultrasound; Sex-linked genetic diseases; Prenatal diagnosis.

## INTRODUCTION

The determination of fetal sex in the early stages of pregnancy has long been a subject of medical interest and research. This interest is not merely academic; it has practical implications for the clinical management of sex-linked genetic disorders and for families with a history of such conditions [1]. Traditional methods of fetal sex determination, such as ultrasound, typically become reliable only in the second trimester, around the $18^{th}$ to $22^{nd}$ week of pregnancy. However, advancements in medical imaging and diagnostic techniques have opened the door to earlier determination, potentially revolutionizing prenatal care and the management of pregnancy [2].

The concept of using anogenital distance (AGD) as a biomarker for early fetal sex determination presents an innovative approach in the field of obstetrics and gynecology. AGD—the measure between the anus and the genitalia—is a parameter that has been shown to differ between males and females postnatally, with implications for reproductive health [3,4]. Extending this metric to prenatal development could provide a non-invasive, early, and reliable method for gender identification. Such a method would be particularly valuable for expecting parents and clinicians dealing with pregnancies at risk of sex-linked diseases, where early diagnosis can significantly influence treatment decisions and outcomes [5].

Despite the potential benefits, the application of AGD for fetal sex determination during the first trimester remains underexplored. Several factors contribute to this gap in research, including technical challenges in measuring AGD accurately during the early stages of pregnancy and the need for standardized measurement protocols. Furthermore, ethical considerations surrounding early sex determination necessitate careful consideration, particularly in terms of consent and the use of this information [6].

The feasibility and reliability of AGD measurement for this purpose also require empirical validation. Previous studies have primarily focused on the anatomical and physiological significance of AGD in animals and humans, with less emphasis on its prenatal diagnostic potential. Establishing a clear, evidence-based understanding of AGD's applicability in early fetal sex determination could significantly impact clinical practice and patient care [7-9].

In light of these considerations, this study aims to investigate the use of AGD as a reliable marker for determining fetal sex in the first trimester of pregnancy.

## PATIENTS AND METHODS

This prospective cohort study has been conducted on 245 pregnant women admitted at the Department of Obstetrics and Gynecology, at Benha University Hospitals (Feto-maternal Unit) From August 2021 to December 2022.

**Inclusion criteria** were women between the ages of 18 and 35, women who were pregnant with a single fetus, gestational age was 11-13 weeks +6 days.
Exclusion criteria were patients who refused to provide consent and multifetal gestation.



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**Methods of measurement:**

The study included a total of 245 pregnant women who attended at Benha University Hospitals at outpatient clinics. These women were selected based on our inclusion criteria during their routine prenatal visits. Following their initial assessment and enrollment in the study at the outpatient clinics, participants underwent further evaluations and follow-ups as part of the study protocol. The study did not involve hospital admission for all participants; instead, follow-up visits were scheduled at the outpatient clinics to monitor their health and pregnancy progress.

All the mothers underwent ultrasound examination conducted by the same ultrasound team. The examination included assessment of fetal AGD in a neutral setting, avoiding hyperflexion or hyperextension, on the midsagittal plane. The images were utilized to ascertain the crown-rump length (CRL) for precise gestational age determination. The AGD was quantified from the caudal fetus to the genital appendage's inferior end. The used ultrasound instrument was mindrayDC-70Exp (North America).

**Grouping:**

The participants were categorized into three groups according to the gestational age: Group 1: women with 11 to 11 weeks, 6 days pregnancy (n=80), Group 2: women with 12 to 12 weeks, 6 days pregnancy (n=80) and Group 3: women with 13 to 13 weeks, 6 days pregnancy (n=85).

The Receiver operating characteristics curves (ROC) were utilized in delineating cut-off for AGD in all groups. The gender of all participants' babies was documented and was followed up until second trimester and after delivery to ensure fetal gender determination.

**Ethical considerations:**

**The study was done after being accepted by the Research Ethics Committee, Benha University. Written informed consent of all the participants was obtained after being informed about the study's requirements, objectives, and potential risks. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring the protection of their confidentiality and privacy. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

**Sample size:**

Assuming the total number of women attending the BUH was 1000 women and positive predictive value anogenital distance was 70%. At 95 CI, and effect size=1, the estimated sample would be 245 pregnant women.

***Statistical analysis***

Data were analyzed with the SPSS software (IBM Corporation, v. 20.0, Armonk, NY). Frequencies and percentages were utilized to express qualitative data. Mean ± standard deviation was utilized to express the quantitative data. Diagnostic performance of AGD in expecting fetal gender was evaluated with ROC curves. The optimal cut-off scores were selected to optimize the sum of specificity and sensitivity. $P<0.05$ was considered significant.

**RESULTS**

The age of males and females did not significantly differ between all groups. However, the AGD was markedly longer in male fetuses compared to female fetuses (**Table 1).**

**Table (1): Participants characteristics:**

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| **Age** | 11w to<br>11w+6d<br>Mean<br>11w+4d | 12w to<br>12w+6d<br>Mean<br>12w+3d | 13w to<br>13w+6d<br>Mean<br>13w+3d |
| **Gender** | Male= 39<br>(48.75%)<br>Female =41<br>(51.25%) | Male= 41<br>(51.25%)<br>Female = 39<br>(48.75%) | Male= 52<br>(61.18%)<br>Female =33<br>(38.82%) |
| **GD (mm)** | 4.59±0.73 | 5±0.87 | 5.1±0.39 |

w: week, AGD: anogenital distance.

**Diagnostic performance of AGD for detecting gender (Table 2):**

The optimal cut-off for detecting gender by AGD was calculated separately in every group to avoid the influence of age on AGD.

**Group 1:**

When optimizing the combined specificity and sensitivity, ROC curve analyses revealed that the optimal cut-off value to predict gender in group 1 was 4.5 mm, the area under the curve (AUC) attained 0.967. Utilizing the 4.5 mm cut-off, fetal gender was accurately established via ultrasound in 100 % of male fetuses (sensitivity) and within 82.9 % of female fetuses (specificity). The possibility to be male with an AGD ≥4.5 mm was 84.87 % (positive predictive value) and the possibility to be female with an AGD <4.5 mm was 100 % (negative predictive value).

**Group 2:**

While the optimal cut-off value to predict gender in group 2 attained 4.5 mm, the AUC attained 0.988. Utilizing the 4.5 mm cut-off, fetal gender was accurately established via ultrasound in 100 % of male fetuses and in 84.6 % of female fetuses. The possibility to be male with an AGD ≥4.5 mm was 87.23% and the possibility to be female with an AGD <4.5 mm was 100%.

**Group 3:**

The optimal cut-off value to predict gender in group 3 attained 4.9 mm, the AUC was 0.928. Utilizing the 4.5 mm cut-off, fetal gender was accurately established via ultrasound in 94.23 % of male fetuses and in 81.82 % of female fetuses. The possibility to be male with an AGD ≥4.9 mm was 89.09 and the possibility to be female with an AGD <4.9 mm attained 90%.

**Table (2): Characteristics and sensitivity of AGD**

| Cut-off | AUC | P | Sensitivity | Specificity | LR | PV+ | PV- |
|---|---|---|---|---|---|---|---|
| 4.5 | 0.967 | <0.001 | 100% | 82.9% | 5.86 | 84.87% | 100% |
| **Group 2** | | | | | | | |
| **4.5** | 0.988 | <0.001 | 100% | 84.6% | 6.5 | 87.23% | 100% |
| **Group 3** | | | | | | | |
| **4.9** | 0.928 | <0.001 | 94.23% | 81.82% | 5.18 | 89.09% | 90.00% |

AGD: Anogenital Distance, AUC: Area Under the Curve, LR: Likelihood Ratio, PV+: Positive Predictive Value, PV-: Negative Predictive Value.

Gender of all cases in each group was detected at second trimester and was ensured after delivery as shown in **table 3.**

**Table (3): Gender of all cases in each group as detected at second trimester and ensured after delivery**

| | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | **Male = 39** | **Female =41** | **Male= 41** | **Female =39** | **Male= 52** | **Female=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 |

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## CASES

**Case 1:** A 33-year-old woman, gravid 3, para 2, at 11 weeks and 6 days gestation as determined by crown-rump length, with an anogenital distance of 4.1 mm; follow-up confirmed the fetus's gender as female.


**Figure 1:** By follow up gender was female. A 33-year-old woman, gravid 3, para 2, at 11 weeks and 6 days gestation as determined by crown-rump length, with an anogenital distance of 4.1 mm; follow-up confirmed the fetus's gender as female.

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**Case 2:** A 28-year-old woman, gravid 2, para 1, at 13 weeks and 1 day gestation, also determined by crown-rump length, with an anogenital distance of 5.1 mm; subsequent follow-up identified the fetus's gender as male.


**Figure 2:** By follow up gender was male. A 28-year-old woman, gravid 2, para 1, at 13 weeks and 1 day gestation, also determined by crown-rump length, with an anogenital distance of 5.1 mm; subsequent follow-up identified the fetus's gender as male.

## DISCUSSION

Early fetal sex determination is of significant importance for managing pregnancies, especially for expectant parents and in cases with a high risk of sex-linked genetic diseases [10]. The use of anogenital distance (AGD), a novel sonographic parameter that measures the distance between the anus and the genital tubercle, offers a promising approach for early gender identification[11]. AGD, which exhibits sexual dimorphism influenced by hormonal environment—typically longer in males than in females—serves as the basis for this study's exploration into the accuracy of fetal sex prediction in the first trimester. Conducted at Benha University Hospitals, this prospective cohort study involved 245 pregnant women, aiming to validate the efficacy of AGD measurement for fetal gender determination early in pregnancy, potentially impacting clinical practices related to prenatal diagnostics and the management of sex-linked disorders. These findings are consistent with previous research that explored the utility of AGD in early fetal sex determination. **Alfuraih *et al.*** [5] conducted a retrospective cohort study on 313 singleton pregnancies within the 11 to 13+6 weeks gestational range, aiming to utilize sonographic estimates of AGD for gender prediction. Their results showed that male fetuses had longer AGD measurements by approximately 14.8% compared to females, with significant variations in AGD observed across different gestational weeks. However, the optimal cut-off point identified through ROC analysis suggested a relatively low sensitivity and specificity, indicating that AGD measurement alone might not offer high accuracy for fetal gender prediction.

Similarly, **Najdi *et al.*** [1] and **Arfi *et al.*** [12] provided evidence supporting the predictive value of AGD for determining fetal sex in the first trimester. **Najdi *et al.*** [1] segmented their study population into three groups based on gestational age, finding significant differences in AGD between male and female fetuses, with the greatest accuracy observed in pregnancies beyond 12 weeks using a cut-off value of 4.9 mm. **Arfi *et al.*** [12] focused on establishing a reliable AGD cut-off for gender determination, identifying 4.8 mm as the threshold that most accurately forecasted the fetal sex. These studies collectively underscore the potential of AGD as a marker for early sex determination, although they also highlight the challenges in achieving optimal diagnostic precision.

In the study by **Sipahi *et al.*** [13], 111 patients with singleton pregnancies between 11 and 13+6 days were examined to assess the accuracy of first-trimester fetal gender determination using AGD measurements. The study was stratified into three groups based on gestational age, revealing that gender determination accuracy varied, with a notable increase in precision for female fetuses in the earliest gestational group and high accuracy for both genders as gestation progressed. Specifically, an AGD threshold of 4.8 mm significantly differentiated male from female fetuses, achieving high specificity and sensitivity rates. This study's strengths lie in its prospective design and focused approach within a single tertiary care center, suggesting AGD's potential as a viable method for early gender determination.

However, the study's limitations, including its smaller sample size and lack of multicenter involvement, might affect the generalizability of its findings. The absence of correlation with crown-rump length (CRL) for gender prediction could also impact the overall predictive accuracy of AGD. Despite these constraints, the research underscores the need for further, larger-scale studies to validate AGD's efficacy in predicting fetal gender, especially during the first trimester. This calls for a broader exploration to overcome the mentioned limitations and confirm AGD's utility in clinical practice.

## CONCLUSIONS

AGD measurement in the first trimester presents a highly accurate, non-invasive method for early fetal sex determination. This technique holds significant promise for advancing prenatal care, particularly in the management of sex-linked genetic disorders, by enabling timely and safer diagnostic interventions.

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