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The value of three-dimensional ultrasound in identifying Mullerian anomalies at risk of adverse pregnancy outcomes

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ABSTRACT

Objective: To examine the reliability of three-dimensional ultrasound (3D-US) in the differentiation between subseptate and arcuate uteri, due to the different associated pregnancy outcomes; refine the existing 3D-US parameters and evaluate the concordance between 3D-US and MRI in diagnosing these anomalies.

Study design: This was a prospective cohort study of 455 women suspected of having a Mullerian anomaly. The diagnosis of subseptate, bicornuate or arcuate uterus was made by 3D-US in 55 women. Two independent examiners manipulated the 3D-US volume datasets and recorded the internal intercornual distance, indentation length, indentation tip angle, and myometrial wall thickness in the coronal plane of the uterus. Subsequently, 48 women underwent MRI which was used as the reference test for diagnosis. We calculated the degree of correlation between the two ultrasound assessors' 3D-US measurements using interclass correlation coefficient and as well as a Bland-Altman plot. The mean values of the four parameters were used to create receiver operating characteristic curves for determining the best cutoff values for differentiation between subseptate and arcuate uteri. We used the Cohen's Kappa test to measure the level of agreement between 3D-US and MRI.

Results: There was good interobserver agreement between the two 3D-US assessors for all four parameters. There was a substantial level of agreement between 3D-US and MRI in differentiating between bicornuate, subseptate and arcuate uteri with a kappa value of 0.727 (95% CI 0.443–0.856). Distinction between subseptate and arcuate uterus was improved when using an indentation length ≥ 12.5 mm (AUC 0.99) and indentation tip angle ≤ 89.25 degrees (AUC 0.97) as cutoffs for diagnosis but not the internal intercornual distance or myometrial wall thickness.

Conclusion: 3D-US evaluation of the coronal view of the uterus can be relied upon to make a noninvasive, accurate differentiation between subseptate and arcuate uteri. The fundal indentation length and indentation tip angle cut offs of ≥ 12.5 mm and ≤ 88 mm, respectively were found to be most accurate for distinction. Thus, allowing for individualizing pre-pregnancy management plans and patient-informed healthcare choices.

HIGHLIGHTS

- There are no agreed upon criteria for differentiating arcuate from subseptate uteri. Such differentiation is critical for counseling and management due to the substantial difference in pregnancy outcome.
- We aimed to propose cut off values for ultrasound measurements standardized against MRI diagnostic criteria for accurate differentiation between arcuate and subseptate uteri.
- We demonstrated substantial agreement between 3D-US and MRI in differentiating between bicornuate, subseptate and arcuate uteri.
- 3D-US evaluation of the coronal view of the uterus is reliable to make an accurate differentiation between subseptate and arcuate uteri.
- Using the indentation length ≥ 12.5 mm and indentation tip angle ≤ 89.25 degrees as parameters to be measured on the coronal view by 3D-US increases its diagnostic accuracy for distinction between arcuate and subseptate uteri.

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Adverse pregnancy outcome; arcuate; subseptate; Mullerian; 3-D US

1. Introduction

Mullerian anomalies have been associated with increased incidence of adverse reproductive outcomes [1–3]. Investigators attempted to determine the prevalence of Mullerian anomalies with reported rates ranging from 0.6% to 38% [4–7]. This wide variation may be due to the difference in classification systems and diagnostic modalities used. A systematic review of 94 observational studies found a prevalence of Mullerian anomalies of 5.5%, 8.0%, 13.3%, 24.5% in cohorts of fertile, infertile women and those with history of miscarriage or miscarriage and infertility, respectively [8]. They reported that arcuate uteri were commonest in the unselected population (3.9%) but prevalence was not increased in high-risk groups. However, septate uterus was commonest in those with a history of miscarriage (5.3%) and in those with both miscarriage and infertility (15.4%).

Recurrent miscarriage has been one of the most distressing obstetric complications to the patients and clinicians. Authors reported a prevalence of Mullerian anomalies in patients with recurrent miscarriages of 54.5% where septated uteri were found to be the most common anomaly and therefore they recommended that Mullerian uterine anomalies should be systematically assessed in patients with recurrent miscarriage [9].

In twin pregnancies, the presence of a uterine anomaly is associated with an increased risk of cerclage, preterm birth and lower birth weights [10].

MRI is valuable for the diagnosis of Mullerian anomalies with reported accuracy of 70–100% [11–14]. Its accurate tissue characterization enables it to distinguish the fibromuscular septum of a septate/subseptate uterus from the muscular myometrial indentation in cases of a bicornuate or arcuate uterus. Studies have demonstrated that the coronal view on three-dimensional ultrasound (3D-US) produces images comparable to MRI, and therefore similar diagnostic accuracy [15–20]. However, differentiating a septate/subseptate from arcuate uterus remains challenging. Attempts by specialty societies, such as the American Society of Reproductive Medicine (ASRM) [21] and the European Society of Human Reproduction and Embryology/European Society of Gynecologic Endoscopy (ESHRE/ESGE) [22,23], to guide clinicians were done. The ESHRE/ESGE classification [22] excludes the entity arcuate uterus, considering it normal. The ASRM guidelines on uterine septum diagnosis and management states that 3D-US, sonohysterography, and MRI are good noninvasive diagnostic tests for distinguishing a septate, arcuate

and a bicornuate uterus when compared with laparoscopy/hysteroscopy [21]. Nevertheless, there are no universally agreed upon criteria to differentiate arcuate from subseptate uteri resulting in confusing clinicians when devising and counseling for management plans [24,25]. This presents the need for an accurate diagnostic tool to triage the patients who need surgical interventions and those who don't, therefore, optimizing the pregnancy outcome and the cost effectiveness.

We aimed to examine the reliability/reproducibility of 3D-US in the differentiation between subseptate and arcuate uterus, as diagnosed by MRI. We selected these easily confused anomalies with different effects on reproductive performance [8], having no universally accepted diagnostic criteria by 3D-US [21–25]. We also wanted to refine the existing 3D-US indices in an attempt to improve their diagnostic accuracy. A secondary outcome, was to evaluate the concordance between 3D-US and MRI.

2. Patients and methods

2.1. Study design

This was a reliability/agreement observational prospective cohort study. The design and report were based on the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement [26]. The study was approved by the scientific and ethical committee of the Department of Obstetrics and Gynecology (approval number O19007) and all recruited women provided written consent forms.

2.2. Sample size

Due to the observational nature of our study and the lack of availability of prevalence data for arcuate and subseptate uterus based on MRI diagnosis for our population a formal sample size calculation was not attempted. Our sample size was based on a convenience sample of 50 women that could be recruited during the allocated study period. Furthermore, some researchers claim that a sample size of 50 patients is adequate in reliability studies and that increasing the sample size would not significantly improve the reliability precision [27,28].

2.3. Study participants

The study population consisted of women referred to the Maternal-Fetal Ultrasound Unit, Cairo University,

Egypt, between October 2018 and November 2019 with suspected Mullerian anomaly following a hysterosalpingography, hysteroscopy, laparoscopy or 2D-US. Women diagnosed with subseptate, bicornuate or arcuate uterus based on 3D-US (index test) were referred for MRI (reference test). We included women diagnosed with bicornuate uterus on 3D-US to mitigate the risk of excluding women with an arcuate or subseptate uterus that would have been misdiagnosed by 3D-US as bicornuate. Exclusion criteria were the presence of a complete uterine septum reaching the internal cervical os (septate uterus) as they can be reliably diagnosed and distinguished from arcuate uterus by 3D-US, as well as the presence of leiomyomas and adenomyosis on 3D-US which may distort the anatomy. To identify the optimal cutoff values for the most valid parameters we only included women confirmed to have either a subseptate or arcuate uterus on MRI.

2.4. Index test

The 3D-US examinations were performed by three experienced examiners (R.K., S.N. and H.E.) during the mid-luteal phase of the menstrual cycle, using a 5–9 MHz transvaginal volume probe (RIC 5–9D Transvaginal probe, Voluson E10 BT 18, and Voluson 730 PRO, GE Medical Systems, Zipf, Austria). Women with irregular cycles were examined on any non-menstrual day. The transvaginal transducer angle was adjusted to visualize the whole uterus in a mid-sagittal position, magnified to occupy 75% of the image. The 3D volume was, then, acquired slowly with a sweep angle of 120 degrees. Two independent examiners, blinded to each other's initial findings, manipulated the 3D volume datasets off-line (R.K and S.N. The coronal plane of the uterus was obtained by manipulating the 3-D datasets to produce a standardized multiplanar view (SMV) of the uterus using the published technique by Martins et al. [29]. The coronal plane of the uterus was examined using the multiplanar mode by applying static volume contrast imaging (VCI static) with a slice thickness set at 2 mm to enhance image contrast. The image was then adjusted to visualize the external contour of the uterus and the endometrial cavity outline including both uterine cornua. Four measurements were recorded: the internal intercornual distance (transverse distance between the two uterine cornu); the indentation length (the vertical length of a line drawn bisecting internal intercornual distance to the lowest point of the cavity indentation); the indentation tip angle; and the myometrial

wall thickness (the length of a vertical line drawn bisecting the internal intercornual distance reaching the serosal border of the myometrium).

Once the volume dataset manipulation was complete the provisional 3D-US diagnosis was made jointly by the two assessors using the ASRM 2016 classification [21].

A uterus was considered subseptate if the indentation length was >15 mm and the indentation tip angle was <90 degrees, otherwise considered arcuate. A diagnosis of a bicornuate uterus was made if the diagnostic criteria for subseptate were fulfilled but the external fundal cleft was >10 mm. To measure the depth of the fundal cleft we used the method proposed by Troiano and McCarthy [19]. Briefly, a line was drawn between the two uterine ostia and then from the midpoint of this line another line was drawn to reach the top of the uterine fundus, which represents the depth of the fundal cleft (Figure 1).

2.5. Reference test

An experienced radiologist, blind to the 3D-US diagnosis, performed MRI for all the cases using a standard body coil, 1.5-T MR (Achieva, Philips Medical Systems, Netherland B.V.). With patients in the supine position and immobile, the following sequences were obtained: Coronal oblique T2, SPIR WIs (most important), sagittal T1, T2 WIs and axial oblique T2, SPIR WIs (T1WI: TR 490 ms, TE 10 ms, Flip 12°, FOV 240 × 292, Slice thickness: 5.2/2.6 mm; T2WI: TR 3259 ms, TE 100 ms, Flip 142°, FOV 350 × 288, slice thickness: 5/0 mm; SPIR WI: TR 3000 ms, TE 90 ms, Flip 90°, FOV 210 × 236, slice thickness: 4/0).

All MRI diagnoses were based on ASRM criteria [21]. For subseptate uteri: normal size, showing a flat or subtle fundal indentation measuring less than 1 cm from a line joining the two uterine tubal ostia, an inter-cornual distance <4 cm and an acute indentation tip angle of <90 degrees. Some septa had a fibrous lower component appearing on T2-weighted images as a low-signal-intensity (hypointense) thin band originating from the iso-intense muscular upper part of the septum thus aiding in the diagnosis. In bicornuate uteri the external fundal indentation was more prominent, measuring >1 cm from a line joining the two tubal ostia with only isointense myometrial tissue seen between the two horns on T2 weighted images having an obtuse indentation tip angle >90 degrees. An arcuate uterus: normal size, with an external uterine contour similar to a subseptate uterus and a

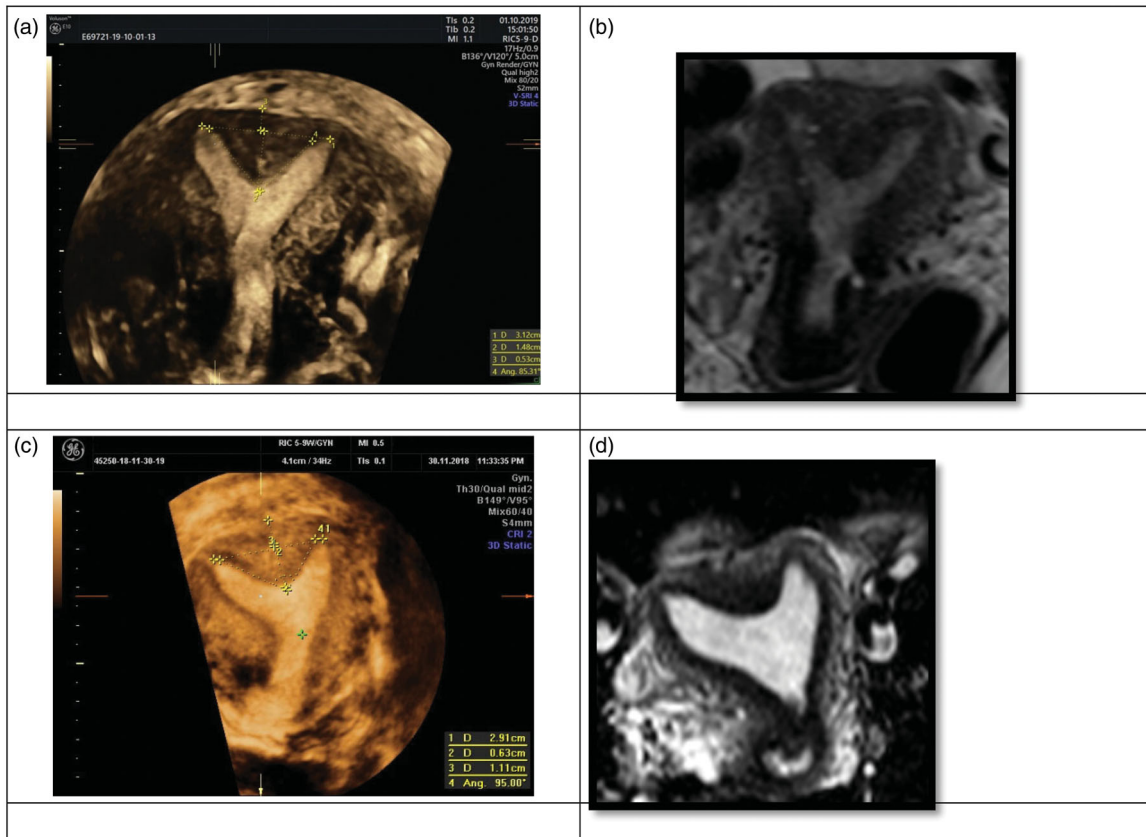


Figure 1. Comparison of three-dimensional ultrasound (3 D-US) using static VCI (with the corresponding distance and indentation tip angle measurements) and magnetic resonance imaging (MRI) of the coronal view of the uterus. Image (a) is a 3 D-US image of a subseptate uterus while image (b) is the same uterus seen by MRI. Image (c) is an arcuate uterus seen by 3 D-US while image (d) is the same uterus on MRI.

broad-based smooth isointense myometrial indentation in the endometrial cavity with no hypointense fibrous tissue, making an angle of more than 90 degrees at the indentation tip (Figure 1).

2.6. Statistical analysis

We assessed the correlation between the two 3 D-US volume dataset assessors for the four parameters using interclass correlation coefficient and presented the relationship using a Bland-Altman plot tested for proportional bias by linear regression analysis. The mean values of the parameters were used to create a receiver operating characteristic (ROC) curve for each to determine the best cutoff value for differentiating subseptate from arcuate uteri based on MRI diagnosis. We used the Cohen's Kappa test to measure the agreement between 3 D-US and MRI in diagnosing arcuate, subseptate and bicornuate uterus. All calculations were done using SPSS version 23 (Statistical Package For The Social Sciences, IBM Corporation, Armonk, NY, USA).

3. Results

We examined 455 women by 3 D-US. 67 of these were provisionally diagnosed to have Mullerian anomalies, including 55 with subseptate, bicornuate or arcuate uterus and 12 with other Mullerian anomalies (7 complete septum, 3 unicornuate uterus, 2 uterus didelphys) with an initial prevalence of only 14.7%. Of the 455 women, only 7 patients had a preliminary diagnosis based on hysteroscopy and 9 on laparoscopy while all 16 patients were found to have Mullerian anomalies on 3 D-US. The remaining patients had their diagnosis based on undocumented 2 D-US examinations (without a report and/or ultrasound images) or poor quality hysterosalpingraphy. The 55 eligible women were offered recruitment into the study but 7 did not consent; the remaining 48 were referred for MRI (Figure 2) (Addendum).

There was good interobserver agreement between the two 3 D-US assessors for all measured 3 D-US parameters (Table 1; Figure 3) (Addendum). The diagnostic performance of 3 D-US in comparison to MRI is

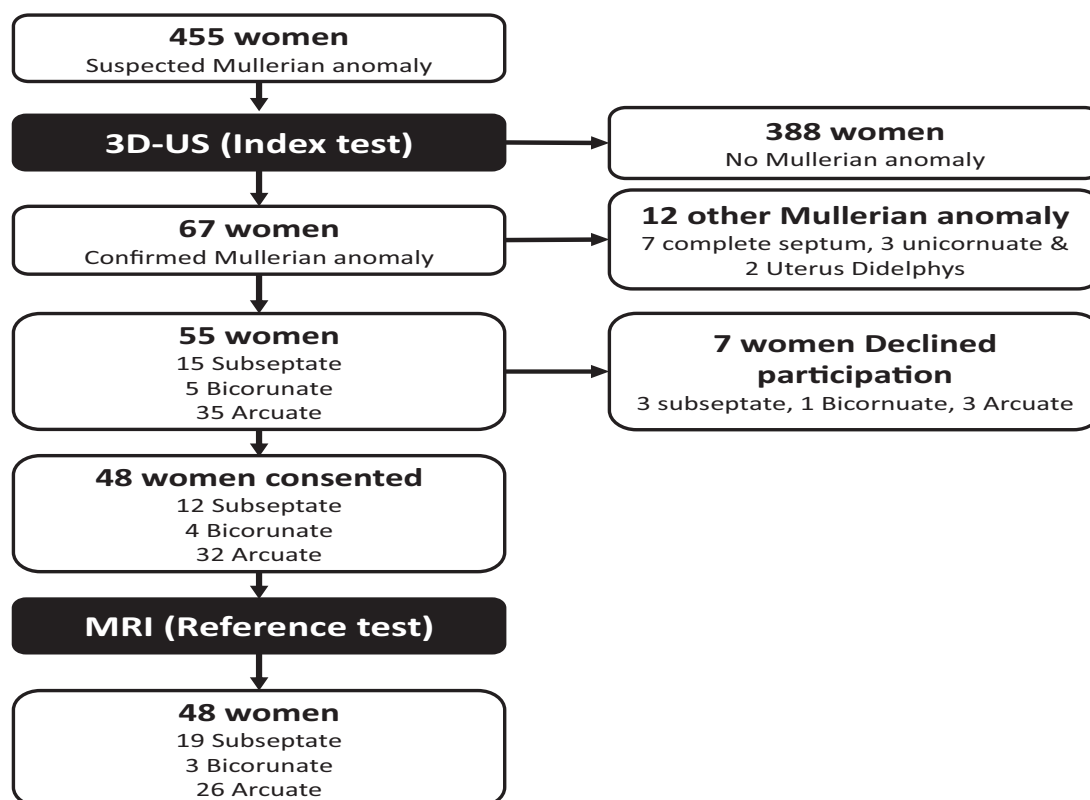


Figure 2. Flow chart demonstrating the flow of participants throughout the study.

Table 1. Interobserver agreement for the measured 3 D-US parameters.

Measurement parameter	Mean (bias)	SD	Difference between the two investigators		Interclass correlation	
			Lower LoA	Upper LoA	<i>r</i>	<i>r</i> ²
Internal intercornual distance	−0.06	1.67	−3.33	3.21	0.9248	0.9606
Indentation length	−0.60	1.66	−3.84	2.65	0.9248	0.8553
Indentation tip angle	−1.09	3.08	−7.12	4.93	0.9635	0.9295
Myometrial wall thickness	0.13	1.02	−1.86	2.12	0.9554	0.9139

SD: Standard deviation; LoA: limit of agreement.

demonstrated in Table 2. There was a substantial level of agreement between both tests in differentiating between bicornuate, subseptate and arcuate uteri with a calculated unweighted kappa value of 0.727 (95% Confidence Interval 0.443–0.856).

The ROC curves for the parameters are presented in Table 3 and Figure 4. Reliable distinction between subseptate and arcuate uterus was provided by an indentation length ≥ 12.5 mm (AUC = 0.99) and an indentation tip angle ≤ 89.25 degrees (AUC = 0.97), but not by the internal intercornual distance or myometrial wall thickness.

4. Discussion

4.1. Main findings of the study

There are three main findings in this study. First, reliable distinction between subseptate and arcuate

uterus can be provided by the 3 D-US measurements of an indentation length ≥ 12.5 mm and indentation tip angle ≤ 89.25 degrees; second, there is very good level of agreement between 3 D-US evaluation of the coronal view of the uterus and MRI in differentiating between bicornuate, subseptate and arcuate uteri; and third, there is good interobserver agreement for the measured 3 D-US parameters.

In this study, we attempted to standardize numerical cutoff values (length in cm and angle in degrees) to differentiate between subseptate and arcuate uteri by 3 D-US based on MRI definitive diagnosis, which we considered the reference imaging modality. We relied on MRI's ability to make a distinction between subseptate and arcuate uterus based not only on measurements but also on the tissue characteristics of a septum as a hypointense structure rather than relying

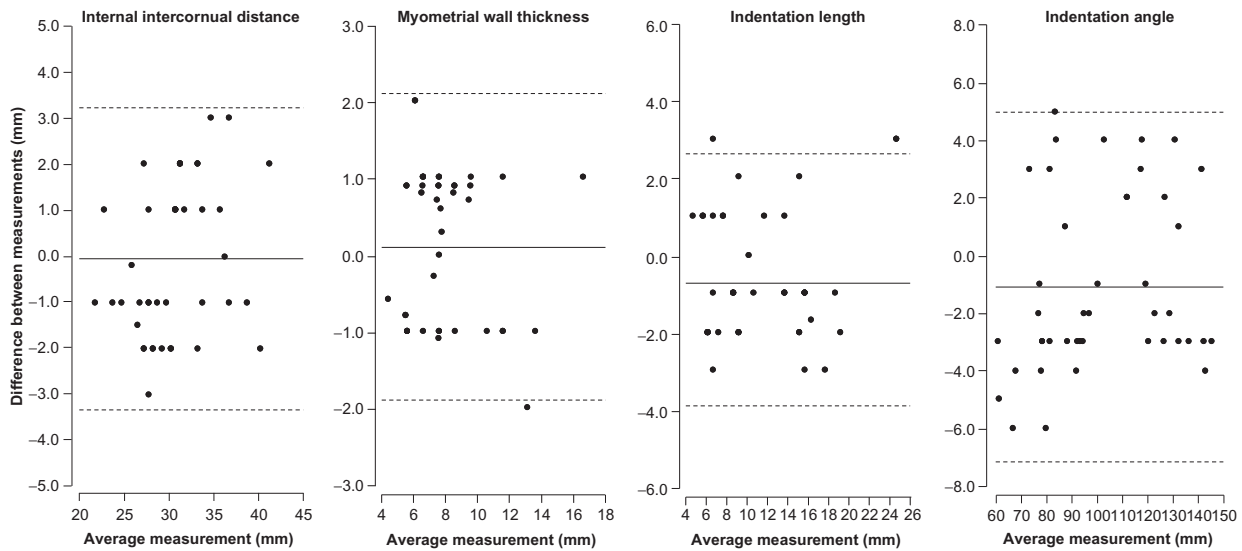


Figure 3. Bland–Altman plots for absolute difference between measurements of two observers.

Table 2. Diagnostic performance of 3 D-US against MRI in differentiating between bicornuate, sub-septate and arcuate uterus.

3D-US	MRI			Total
	Bicornuate	Subseptate	Arcuate	
Bicornuate	2	2	0	4
Subseptate	1	11	0	12
Arcuate	0	6	26	32
Total	3	19	26	48

Unweighted Kappa value: 0.7273, 95% Confidence interval (0.4429–0.8559).

Table 3. The AUC and proposed cutoff values from the constructed ROC curves.

Parameter	AUC	Cutoff value	Sensitivity	False + ve rate
Indentation length	0.99	12.5 mm	100%	3.8%
Indentation tip angle	0.97	91.25 degrees	89.5%	3.9%
Internal intercornual distance	0.48	31.25 mm	73.7%	61.5%
Myometrial wall thickness	0.46	7.47mm	52%	46%

solely on numerical measurements used by 3 D-US. To our knowledge, this has not been previously reported.

4.2. Comparison with results of previous studies

Our finding of a good concordance between 3 D-US and MRI in the diagnosis of Mullerian anomalies is consistent with that of Bermejo et al. [15] who examined 65 women by 3 D-US and MRI and reported a Cohen’s kappa value of 0.88 for concordance between the two diagnostic modalities, however, their study included only three women with arcuate uterus.

We found that there was good inter-observer agreement in the 3 D-US measurements between the two examiners. This high level of agreement and reproducibility of distance and angle measurements

between different observers was also demonstrated by other groups [22,23,25].

Ludwin et al. in a study attempting to distinguish between normal/arcuate and septate uterus assigned two experienced observers to measure indentation depth, indentation angle and indentation to-wall-thickness ratio of coronal 3 D-US images they obtained from 100 women [30]. They assessed the interobserver reliability using the concordance correlation coefficient (CCC) and found a good interobserver reproducibility of indentation depth (CCC, 0.99), indentation angle (CCC, 0.96) and indentation to-wall-thickness ratio (CCC, 0.92). In the same study, Ludwin et al. sent the previously mentioned 100 3 D-US images of the coronal plane of the uterus to 15 experts (five each of clinicians, surgeons and radiologists specialized in gynecological imaging) asking them to indicate whether they believed the uterus in question was arcuate/normal or septate which they defined as a uterus with a clinically relevant degree of distortion caused by the internal indentation [30]. The authors, however did not ask the experts to perform any measurements on the images or indicate what criteria they based their diagnoses upon, but relied solely on their subjective opinion. In our study we attempted to be objective by conducting measurements using the criteria suggested by the ASRM [21] (for both the 3 D-US and MRI assessments) to differentiate between arcuate and subseptate uteri thus making our results more readily reproducible by other researchers and clinicians.

We demonstrated that only the indentation length at a cutoff value of ≥ 12.5 mm and the indentation tip angle at a cutoff value of ≤ 89.25 degrees could

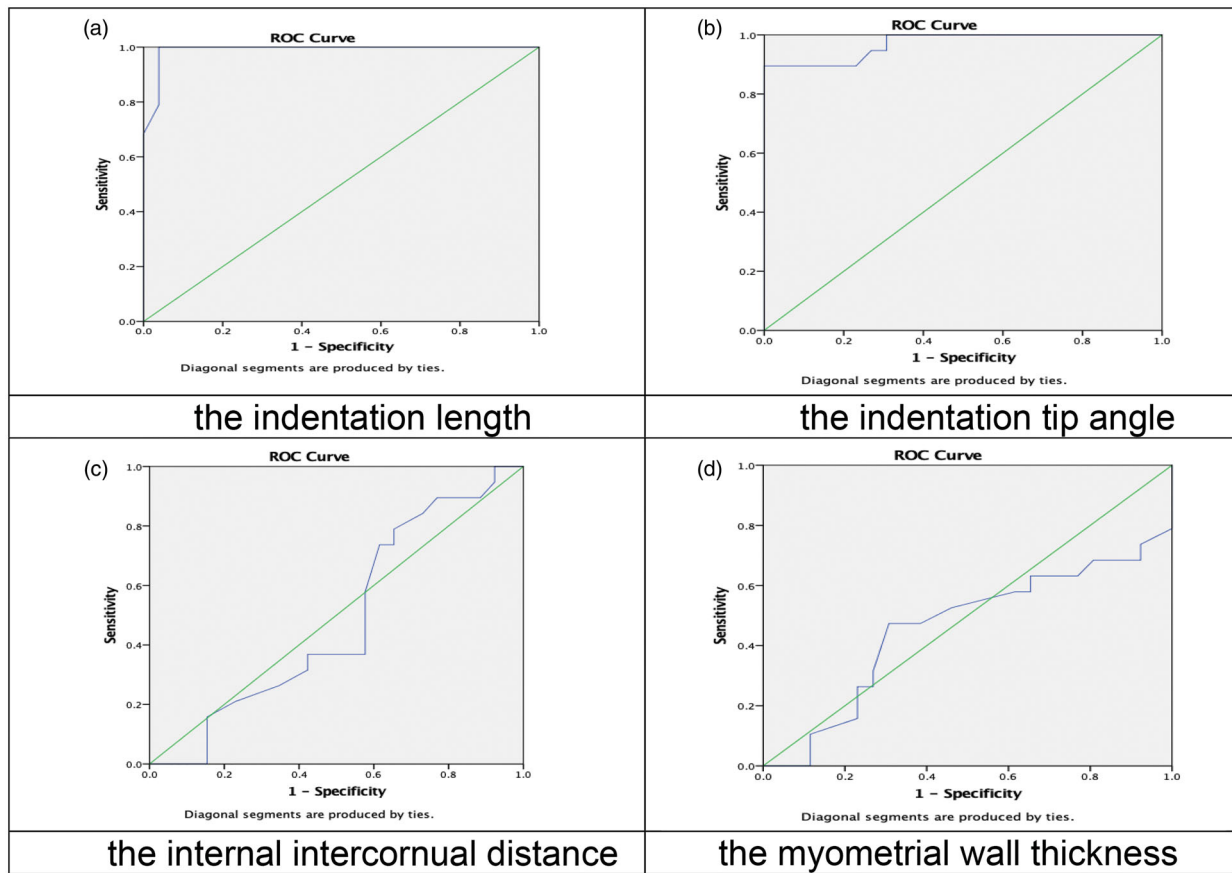


Figure 4. The constructed ROC curves for the four parameters under study.

reliably distinguish between a subseptate and an arcuate uterus. These cutoffs are similar but not identical to those proposed by the ASRM on the diagnosis and management of the septate uterus; the recommended cutoff for indentation length is ≥ 15 mm and for indentation tip angle is ≤ 90 degrees [21].

4.3. Strengths and limitations

Our study has several strengths increasing the generalizability of our findings. First, experienced independent assessors performed and interpreted the 3D-US; this would have minimized heterogeneity in data acquisition, processing and interpretation, which was confirmed by the high level of agreement between the two assessors. Second, we relied on internationally accepted guidelines for 3D-US and MRI diagnoses to reduce the risk of introducing classification bias. Third, we mitigated the risk of diagnostic review bias by ensuring that MRI assessor was blind to 3D-US findings.

The main limitation of the study relates to the relatively small number of cases; however, we accounted for all the women referred to our unit over the study

period with suspected Mullerian anomaly and hence reduced the risk of selection bias. Another limitation is that many of the patients that were referred for 3D-US in our unit were found not to have Mullerian anomalies on initial assessment. This can be explained by the fact that our unit is a tertiary referral center receiving many improperly diagnosed patients due to limitations in training and resources in rural areas.

5. Conclusions

3D-US evaluation of the coronal view of the uterus can be relied upon to make a fairly reliable distinction between a subseptate, arcuate and bicornuate uterus. Further studies using independent data sets are required to assess the accuracy of 3D-US in differentiating subseptate from arcuate uterus when using indentation length and indentation tip angle cut offs of ≥ 12.5 mm and ≤ 88 mm, respectively.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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