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Amniotic sac reference interval in early pregnancy between 7 and 10 weeks' gestation

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CONTRIBUTION

What are the novel findings of this work?

Our study has produced the first comprehensive reference interval for amniotic sac size in early pregnancy which could be used in routine clinical practice.

What are the clinical implications of this work?

Our newly defined reference ranges for the amniotic sac diameter in relation to the crown-rump length and the gestational sac diameter could facilitate earlier and more accurate detection of earlier embryonic abnormalities in the first trimester.

ABSTRACT

Objective – To establish a normal reference interval for amniotic sac measurements between 7 and 10 weeks of gestation and its relative size in relation to the gestational sac and the embryo.

Method – This was a prospective, cross-sectional study of consecutive women presenting to UCLH Early Pregnancy Unit between August 2022 to June 2023. We included live, normally sited, singleton pregnancies with a normal 20-week anomaly scan. We collected 120 cases per gestational week totaling 360 cases. We performed an inter and intra-observer variability assessment in the measurement of mean ASD in 30 patients. Regression analyses were used to establish reference intervals for GSD to CRL, ASD to CRL, GSD to ASD and GSD:ASD ratio to CRL. The fitted regression line was calculated, along with a 90% prediction interval and the R^2 value.

Results – There was good interobserver agreement (difference 0.007mm ± 1.105 (95%CI -2.160 to 2.174)) and good intra-observer agreement between Observer A (0.007 ± 1.105 (-2.160 to 2.174)) and Observer B (-0.014 ± 0.919 (-1.814 to 1.786)) in the measurement of mean ASD in 30 patients. Regression analyses showed a highly statistically significant association between each pair of values (all p-values <0.001). There were significant quadratic associations between mean GSD and CRL (R^2 = 56%) and mean GSD and ASD (R^2 = 60), significant cubic association between ASD and CRL (R^2 = 90%) and significant quadratic association between GSD to ASD ratio and CRL (R^2 = 68%). The regression equations were used to quantify the values of ASD and GSD to ASD ratios for a range of CRL values and gestational age in days.

Conclusion – Our study has produced comprehensive reference intervals for amniotic sac size in early pregnancy which could be used in routine clinical practice.

INTRODUCTION

The amniotic sac develops during the third week after conception from the bilaminar embryonic disc of the implanted blastocyst and subsequently surrounds the developing embryo[1]. At the end of the first trimester the extraembryonic coelom is gradually obliterated by the expanding amniotic sac which fuses with the placental chorionic plate. The amniotic fluid is initially made of embryonic bioproducts diffusing through embryonic skin or through oropharyngeal and cloacal membranes[1]. The fluid electrolyte composition and acid-base balance change rapidly after 10 weeks of gestation reflecting the fetal kidney development from the mesonephros to the metanephros[2]. During the second and third trimester the pool of amniotic fluid is subject to a constant turnover with the accumulation of fetal lung fluid and urine and removal by fetal swallowing[2].

The amniotic cavity becomes visible on transvaginal ultrasound scan (TVS) from seven weeks of gestation i.e. after the last menstrual period[3], yet has been studied comparatively less compared to other early embryonic structures. Robinson and Fleming were the first to establish the normal reference ranges for the first trimester crown-rump length[4], whilst later studies also provided nomograms for the gestational sac (GS) and yolk sac (YS) diameters, and for the embryonic heart rate[5]. However, neither created reference intervals for the amniotic sac. This could be partly explained by difficulties in visualizing the amniotic membrane on transabdominal scan.

Recent studies have shown that the finding of an amniotic cavity which does not contain a live embryo is a reliable diagnostic sign of early miscarriage[6]. Although an abnormally sized exocoelomic cavity and yolk sac could be used to diagnose aneuploid pregnancy and predict

miscarriage[7,8], the potential diagnostic value of assessing the size of the amniotic sac in early pregnancy has not been studied before. Few studies have examined the normal distribution of amniotic sac dimensions, most of which were small and focused on measuring volume[9-13]. The aim of this study was to establish a normal reference interval for amniotic sac measurements between 7 and 10 weeks of gestation and its relative size in relation to the gestational sac and the embryo. This information could be used in the future to examine the value of amniotic sac size measurement for the diagnosis of early pregnancy abnormalities.

METHODS

This was a prospective cross-sectional study conducted at the Early Pregnancy Unit, University College Hospital, London, UK from August 2022 to June 2023. We included consecutive pregnant women with a live normally sited (eutopic) singleton pregnancy with a visible amniotic sac on TVS, from 7 up to 10 weeks' gestation. We included both spontaneous pregnancies and those conceived through ART. We decided on the upper limit of the reference interval of 10 weeks' gestation as from then on transabdominal scans can be carried out to diagnose major fetal structural anomalies and non-invasive prenatal testing for aneuploidies is readily available. Each woman contributed only one pregnancy and a single set of measurements.

We excluded pregnancies resulting in miscarriage, termination of pregnancy and when the outcome of the anomaly scan was unknown. We also excluded multiple pregnancies including those that resulted in a singleton pregnancy due to miscarriage of one twin and pregnancies found to have congenital structural or chromosomal anomalies on the 20-week anomaly scan.

We obtained clinical information as part of routine clinical practice, including maternal age, gravidity, parity, mode of conception, menstrual cycle length and regularity, and indication for presentation[14]. All patients underwent a TVS using high-end ultrasound equipment (Voluson E8, GE Medical Systems, Milwaukee, WI, USA). Ultrasound examinations were performed by clinical fellows, who were all Level II operators[14]. They were supervised by five consultant gynecologists with special interest in early pregnancy care who were all Level III ultrasound operators. The examinations were carried out in a standardized way to ensure that all relevant measurements were performed and recorded.

A live normally sited pregnancy was defined by the presence of a gestational sac within the uterine cavity which contained an embryo with visible cardiac activity.

In all cases the following structures were routinely examined:

- Gestational sac – a spherical structure within the uterine cavity surrounded by echogenic trophoblast.
- Yolk sac – a small spherical structure within the gestational sac.
- Amniotic sac – a spherical thin-walled structure within the gestational sac and distinct from the yolk sac, in which the embryonic pole was situated.

Measurements were obtained using two-dimensional images only, whilst three-dimensional volumes were only used for the assessment of inter- and intraobserver variability. The mean gestational sac diameter (GSD) was calculated as the average of three perpendicular diameters with the calipers placed at the inner aspects of the chorionic cavity[15]. Mean amniotic sac diameter (ASD) was calculated as the average of three perpendicular diameters with the calipers placed at the inner edge of the amnion sac wall (Figure 1). The embryonic heart rate (HR) was calculated as beats per minute (bpm) by using M-mode. Crown-rump length (CRL) was measured in a sagittal section of the embryo[16]. Gestational age was derived from the measurement of CRL using the formula described by Robinson and Fleming[4].

Clinical information and ultrasound images were stored on dedicated hospital clinical databases (GE HealthCare ViewPoint version 5.6.25.283) as per our routine practice and data for the study was collected only by members of the clinical care team. Data was anonymized and securely stored according to General Data Protection Regulations (GDPR).

The primary objectives were to determine reference intervals for mean ASD and GSD to ASD ratio according to CRL. Further analyses added as supplementary data included reference intervals for mean ASD, GSD and embryonic HR according to gestational age as measured from last menstrual period (LMP) or in vitro fertilization (IVF) conception dates, as these may be useful in routine clinical practice.

Assessment of inter- and intraobserver variability

Inter- and intraobserver variability was assessed in a subgroup of 30 patients. Real time examinations were performed, and three-dimensional volumes were obtained. The depth of the acquired volume was adjusted to cover the entire uterus containing the gestational sac. The probe was held steady, and patients were asked to hold their breath while volume acquisition was on. The rendered volumes were saved on the ultrasound machine hard drive, and they were examined independently by two operators (Operator 1 and Operator 2). Three measurements of the amniotic sac were taken in longitudinal and transverse reformatted sections to assess interobserver variability. All measurements were recorded by a third investigator who did not participate in the reproducibility analysis. Operators were therefore blinded to their own and to each other's measurements. The operators were then asked to re-examine the stored three-dimensional volumes and to repeat the measurements after a minimum of one hour from the first examination to test the intra-observer variability The order of examinations was determined randomly by the third investigator to minimize the risk of bias.

The inter- and intraobserver agreement was assessed using the Bland-Altman limits of agreement method, due to the continuous nature of the measurements. This method

measures the size of differences between pairs of values that are likely to occur. The measure was obtained by calculating the difference between repeat measurements for each patient. The 95% limits of agreement (within which 95% of all differences between values should occur) were then calculated according to the equation: mean difference ± 1.96 × (SD of differences).

Sample size

Recommendations suggest that measurements are obtained from a minimum of 120[17] to 200 individuals and at least 20 per gestational week[18,19]. Our aim was to recruit 120 consecutive cases per gestational weeks 7, 8 and 9, amounting to 360 individual patients in total.

Statistical analysis

Statistical analysis of the data was performed using Stata. The baseline variables for normality of distribution were tested using the Shapiro-Wilk test. Normally distributed values are expressed as mean and 95% confidence intervals. Non-normally distributed values are expressed as medians and interquartile ranges. Descriptive data for categorical variables were presented as numbers and percentages. Reference intervals were generated using recommendations by Royston and Wright[20]. All analyses were performed using linear regression. Where the assumptions of linear regression were not met (e.g. non-normally distributed residuals, a fitted value/residual relationship), either one or both variables were analyzed on the log scale. Where required, a small constant was added onto all values before the transformation. The shape of the relationship between variables was examined. Where it improved the fit of the regression models, quadratic and cubic terms for the predictor variable were included in the analysis. The fitted regression line was calculated, along with a 90%

prediction interval. The strength of the relationship between variables was also quantified by calculating the R^2 value.

We were advised by the National Health Service Research Ethics Committee and the Joint Research Office at UCLH that formal ethical approval was not needed for this study as the data was collected as part of routine care, was anonymized, and analyzed within the care team. This study was registered with the Research Registry with the unique identifying number: researchregistry8168.

RESULTS

During the study period, 2038 women presented to the EPU with 2117 pregnancies. A total of 360 live pregnancies between 7+0 to 9+6 weeks' gestation were included into the study. The study flowchart is shown in Figure 2. There was an equal distribution of 120 cases per each week of gestation. Maternal characteristics are shown in Table 1. The CRL was measured in all cases. Three measurements for each the GS and AS were also recorded in all cases but embryonic heat rate was missing in four cases. Of the 331/360 (92%) cases with a known final pregnancy outcome, no aneuploidies were diagnosed at birth.

Inter- and intraobserver variability in amniotic sac measurements

Inter- and intraobserver agreement in the measurement of mean ASD in 30 patients is summarized in Table 2. There was good interobserver agreement, reflected in a small average difference between the repeat measurements. The difference between the measurements obtained by the two observers, and those obtained by Observer A and B at two different time points (intraobserver agreement) were dispersed around the mean value and also showed

good agreement. Bland-Altman plots with 95% limits of agreement for inter- and intraobserver variability are displayed in Figure 3.

Regression equations

The relationship between each pair of variables was fitted. A summary of the results is given in Table 3. For each relationship, all regression coefficients are reported, along with corresponding 95% confidence intervals, p- and R^2 values.

The results suggested that there was a highly statistically significant association between each pair of variables (all p-values <0.001). Although all relationships were highly statistically significant, some relationships were stronger than others. The strongest relationship was between mean ASD and CRL where the R^2 value was 90%.

There were significant quadratic associations between mean GSD and CRL (R^2 = 56%) and mean GSD and ASD (R^2 = 60) with regression lines and 90% prediction intervals shown in Figures 4 and 5. There was a significant cubic association between ASD and CRL (R^2 = 90%) with regression lines and 90% prediction intervals shown in Figure 6. There was a significant quadratic association between GSD to ASD ratio and CRL (R^2 = 68%) with regression lines and 90% prediction intervals shown in Figure 7.

Regression lines and 90% prediction intervals for mean GSD, ASD and GSD to ASD ratio according to GA in days as calculated by LMP or IVF dates are shown in Table S1 and Figures S1-S3.

The regression equations were also used to quantify the values of ASD and GSD to ASD ratios for a range of CRL values and GA in days, shown in Tables 4 and 5.

DISCUSSION

Main findings

We have collected sufficient data to establish reference intervals for amniotic sac size in live normally sited (eutopic) pregnancies between 7- and 10-weeks' gestation. In all pregnancies the amniotic sac was clearly visualized, and all measurements were successfully and accurately completed with a good inter- and intra-observer variability. We have also provided charts showing mean ASD, and GSD to ASD ratios according to CRL and GA, which are easy to use and can be incorporated into routine clinic practice.

Strengths & limitations

Our study followed stringent methodological quality criteria[21] to develop a high-quality biometric reference interval for amniotic sac measurements in early pregnancy. We assessed the association of parameters to CRL as previous reports recommend the use of CRL for establishing GA-related reference intervals, rather than LMP which is subject to variability and irregularity of menstrual cycles and uncertainty of menstrual dates[5]. We only included pregnancies with a live outcome without congenital structural or chromosomal abnormalities up to the 20-week anomaly scan. There was one case that was a consistent outlier due to a larger mean GSD, but the nuchal scan findings were normal, and the patient had a healthy baby at term. In view of that this case was not excluded from the reference interval as advised by Altman and Chitty[22]. A potential limitation of our study is the inclusion of symptomatic patients, although we only reviewed pregnancies with a live outcome. We also had incomplete follow up of patients after 20 weeks' gestation. However, the potential benefits of establishing the reference range for ASD between 7 to 10 weeks' are better assessment of the risk of miscarriage in live pregnancies and improved early detection of embryonic

anomalies including aneuploidies prior to routine first trimester anomaly scans which can be performed from 10 weeks' onwards[23].

Our study included both ART and spontaneously conceived pregnancies. Previous studies have reported both smaller and larger CRL in ART pregnancies compared to spontaneously conceived pregnancies[24-27]. A systematic review has highlighted both underestimation and overestimation of CRL measurements between assisted and spontaneous conceptions leading to conflicting results in very early pregnancies of <51days[28]. As we only included pregnancies 7-10 weeks' gestations these observed variations should not have a bearing on the accuracy of CRL measurements in our study.

We found a highly significant positive association between mean ASD and CRL with an R^2 value of 90%. At 7-9 weeks of gestation this is likely due to the accumulation of early embryonic bioproducts[1] whereas from 9 weeks of gestation the rapid expansion of the amniotic cavity is associated with the development of nephrons and definitive kidneys[2]. This explains the reducing GSD to ASD ratio as the CRL increases (R^2 value = 68%). An increase in amniotic fluid as CRL increases indicates a normal embryonic development during organogenesis and a discrepancy could be a sign of an underlying embryonic abnormality. Oligohydramnios with amnio-chorionic separation in the second trimester has been reported to be associated with triploidy, fetal congenital anomalies and major structural malformations such as renal agenesis, pulmonary airway malformation and cardiac anomalies[29-31]. There are few anomalies which can be detected in the first trimester, amongst them body stalk anomaly (BSA). This anomaly is typically associated with abnormalities of the amniotic membrane and routine examination of the amniotic sac could facilitate its early detection[32]. Literature on congenital anomalies and associated oligohydramnios in the first trimester, however, is

sparse[33,34] highlighting future research potential and the clinical relevance of establishing reference intervals.

Ratios of head and trunk measurements in the first trimester have been used to predict aneuploidies[35]. In our practice, we have seen pregnancies with selective reduction of the exocelomic cavity and low GSD to ASD ratio which were associated with chromosomally abnormal pregnancies. The relationship between GSD to ASD ratio and CRL in our study showed a narrow 90% interval range which could be used to detect disproportions in sizes of the two compartments. This could facilitate earlier detection of abnormal pregnancies and facilitate better counselling of patients.

Various studies have reported on the roles of the gestational sac, yolk sac, CRL and embryonic cardiac activity biometry to predict adverse outcomes in early pregnancy[36-40]. Other studies have reviewed a combination of ultrasound and demographic variables to create risk prediction models to predict pregnancy outcome[8,41-43]. None of the proposed models so far has reached the level of accuracy for the prediction of miscarriage that is required for the use in routine clinical practice. There has been no research looking at the discrepancies in the size of early pregnancy amniotic fluid compartments to predict adverse outcomes.

Advances in technology and the use of TVS for greater resolution power enables detection and accurate measurement of the amniotic sac, as demonstrated in our study. The few studies on first trimester amniotic sac biometry have mostly focused on assessing the amniotic fluid volumes (AFV). Weissman et al., 1996 measured AFV in 95 pregnant women using two-dimensional ultrasound and the ellipsoid model[9], and found good correlation between GA (measured in weeks) and AFV (r^2 = 78%). Similarly, smaller studies using three-dimensional

volumetry found a significant association between ASV and GA, and ASV and CRL[10-12]. However, three-dimensional imaging may be less accessible and more complex than two-dimensional methods and appears to add little to the diagnostic and prognostic value of two-dimensional imaging[44,45]. Others argue that sections reconstructed from ultrasound volumes may be less accurate than two-dimensional measurements[13]. The ASV interval also widens as the pregnancy advances, making it less valuable in detecting discrepancies. One study of women between 5 and 12 weeks of gestation, of which 193 had an amniotic sac, found a significant correlation between ASD and GA (R^2 = 74%) and CRL (R^2 = 90%), which was similar to our study (GA R^2 = 79%, CRL R^2 = 90%), although they did not specify how ASD was measured or whether this was a single measurement or mean[13].

Implications for clinical practice and future research

The amniotic sac is not routinely measured in early pregnancy, possibly due to lack of evidence demonstrating its clinical relevance in the prediction or diagnosis of early pregnancy complications. Previous studies, however, have shown that the finding of an amniotic sac on ultrasound in the absence of a live embryo is an accurate predictor of miscarriage[46,47] with one large prospective study reporting this finding to have 100% specificity and 100% positive predictive value for pregnancy failure[6]. Close examination of the amniotic sac can also help detect anomalies incompatible with life, such as BSA, in early pregnancy. Further studies are needed to determine whether amniotic sac size discrepancies or new algorithms can reliably predict adverse pregnancy outcomes. We have demonstrated the ease and accuracy of measuring the amniotic sac and it should be incorporated into routine early pregnancy ultrasound assessments.

Conclusion

Our study has produced comprehensive reference intervals for amniotic sac size in early pregnancy which could be used in routine clinical practice. Future research is needed to investigate the potential diagnostic value of discrepancies in size of amniotic and coelomic cavities for the prediction of miscarriage and early detection of embryonic and fetal anomalies.

DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

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FIGURE LEGENDS

Figure 1: Measurement of the amniotic sac in three planes (a, b)

Figure 2: Study flowchart

Figure 3: Bland-Altman plots with 95% limits of agreement for inter- (a) and intraobserver variability (b, c)

a) Interobserver variability of ASD measurement (mean difference 0.007 mm, 95% limits of agreement (-2.160, 2.174)
b) Intraobserver variability of ASD measurement for observer A (mean difference -0.080 mm, 95% limits of agreement (-1.532, 1.372)
c) Intraobserver variability of ASD measurement for observer B (mean difference 0.014 mm, 95% limits of agreement (-1.814, 1.786)

Figure 4: Fitted relationship between GSD and CRL (regression line and 90% prediction interval)

Figure 5: Fitted relationship between GSD and ASD (regression line and 90% prediction interval)

Figure 6: Fitted relationship between ASD and CRL (regression line and 90% prediction interval)

Figure 7: Fitted relationship between GSD:ASD ratio and CRL (regression line and 90% prediction interval)

TABLES

Table 1: Demographic characteristics of 360 women with live pregnancies of 7-9+6 weeks gestation

Characteristic		**N[+]= 360 (%)**
Median maternal age* (Q1-Q3)		33 (30-36.0)
Median gestational age[#], (Q1-Q3)		59 (54-64)
Gravidity	1	92 (25.6)
	2	97 (26.9)
	3	86 (23.9)
	4+	85 (23.6)
Parity	0	192 (53.3)
	1	104 (28.9)
	2+	64 (17.8)
Mode of conception	Spontaneous	321 (89.2)
	ART	39 (10.8)
Indication for presentation	Abdominal pain only	87 (24.2)
	Vaginal bleeding only	48 (13.3)
	Pain & bleeding	137 (38.1)
	Reassurance	66 (18.3)
	Other	22 (6.1)

[+]N is the total frequency unless otherwise stated. Each entry is the observed frequency (percentage), *years, [#]days ART = artificial reproductive therapy

Table 2: Inter- and intraobserver variability for measurement of mean amniotic sac diameter

Agreement	**Difference (mm), SD, 95%CI**
Interobserver	0.007 ± 1.105 (-2.160 to 2.174)
Intraobserver (Observer A)	-0.080 ± 0.741 (-1.532 to 1.372)
Intraobserver (Observer B)	-0.014 ± 0.919 (-1.814 to 1.786)

Table 3: Summary of regression equations according to CRL

Outcome	Predictor	Term	Coefficient (95% CI)	P-value	R^2
Mean GSD	CRL	Constant	10.3 (5.2, 15.3)	<0.001	56%
		Linear	1.51 (0.95, 2.08)		
		Quadratic	-0.017 (-0.032, -0.002)		
Mean ASD	CRL	Constant	10.5 (2.0, 19.0)	<0.001	90%
		Linear	-1.28 (-2.74, 0.17)		
		Quadratic	0.13 (0.06, 0.21)		
		Cubic	-0.002 (-0.004, -0.001)		
Mean GSD	Mean ASD	Constant	15.1 (12.1, 18.1)	<0.001	60%
		Linear	1.15 (0.82, 1.48)		
		Quadratic	-0.011 (-0.019, -0.003)		
GSD:ASD ratio (*)	CRL	Constant	1.73 (1.55, 1.91)	<0.001	68%
		Linear	-0.083 (-0.104, -0.063)		
		Quadratic	0.0011 (0.0006, 0.0017)		

(*) Variable analysed on the log scale (base e)

ASD = amniotic sac diameter, CRL = crown-rump length, GSD = gestational sac diameter

Table 4: Estimated ASD and GSD:ASD ratio according to CRL

CRL	Predicted ASD (mm)			Predicted GSD:ASD		
(mm)	5th	50th	95th	5th	50th	95th
8	3.6	7.7	11.7	2.35	3.11	4.13
9	4.2	8.2	12.1	2.21	2.92	3.87
10	4.9	8.8	12.7	2.08	2.75	3.63
11	5.7	9.6	13.5	1.96	2.59	3.42
12	6.6	10.5	14.4	1.85	2.44	3.23
13	7.6	11.5	15.4	1.75	2.31	3.05
14	8.7	12.6	16.5	1.66	2.19	2.90
15	9.8	13.7	17.7	1.58	2.09	2.75
16	11.1	15.0	18.9	1.51	1.99	2.62
17	12.3	16.2	20.1	1.44	1.90	2.51
18	13.6	17.5	21.5	1.38	1.82	2.40
19	15.0	18.9	22.8	1.32	1.74	2.30
20	16.3	20.2	24.1	1.27	1.68	2.21
21	17.6	21.5	25.4	1.22	1.62	2.13
22	18.9	22.8	26.7	1.18	1.56	2.06
23	20.2	24.1	28.0	1.14	1.51	1.99
24	21.4	25.3	29.2	1.11	1.47	1.93
25	22.6	26.5	30.4	1.08	1.42	1.88
26	23.6	27.6	31.5	1.05	1.39	1.83

27	24.6	28.5	32.5	1.03	1.36	1.79
28	25.5	29.4	33.3	1.00	1.33	1.76
29	27.4	31.6	35.8	0.94	1.25	1.66
30	27.2	31.3	35.4	0.97	1.28	1.70
31	26.8	30.8	34.8	0.93	1.24	1.65
32	27.4	31.8	36.2	0.98	1.30	1.72
33	26.2	30.2	34.1	0.95	1.26	1.68

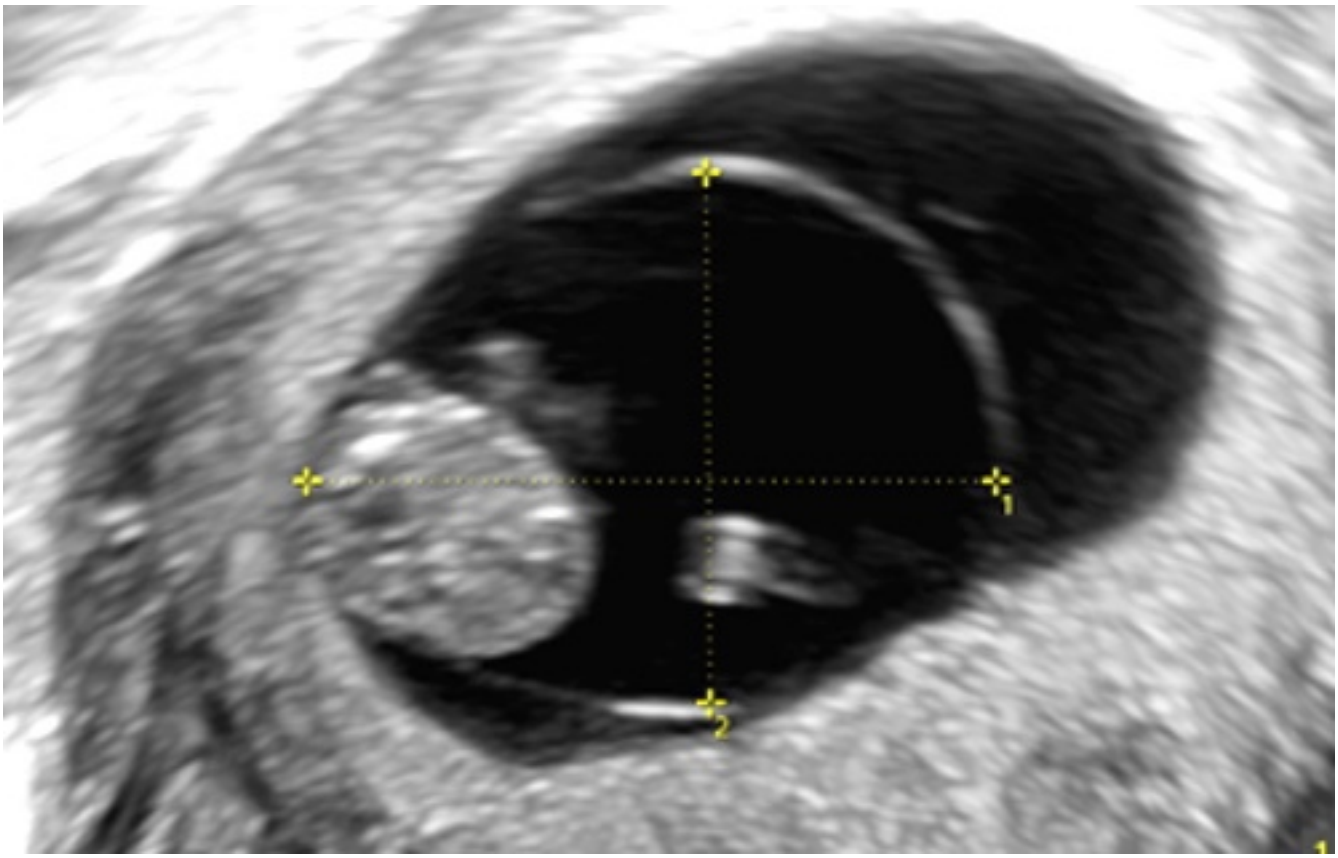
ASD = amniotic sac diameter, CRL = crown-rump length, GSD = gestational sac diameter

Table 5: Estimated ASD and GSD:ASD ratio according to GA in days

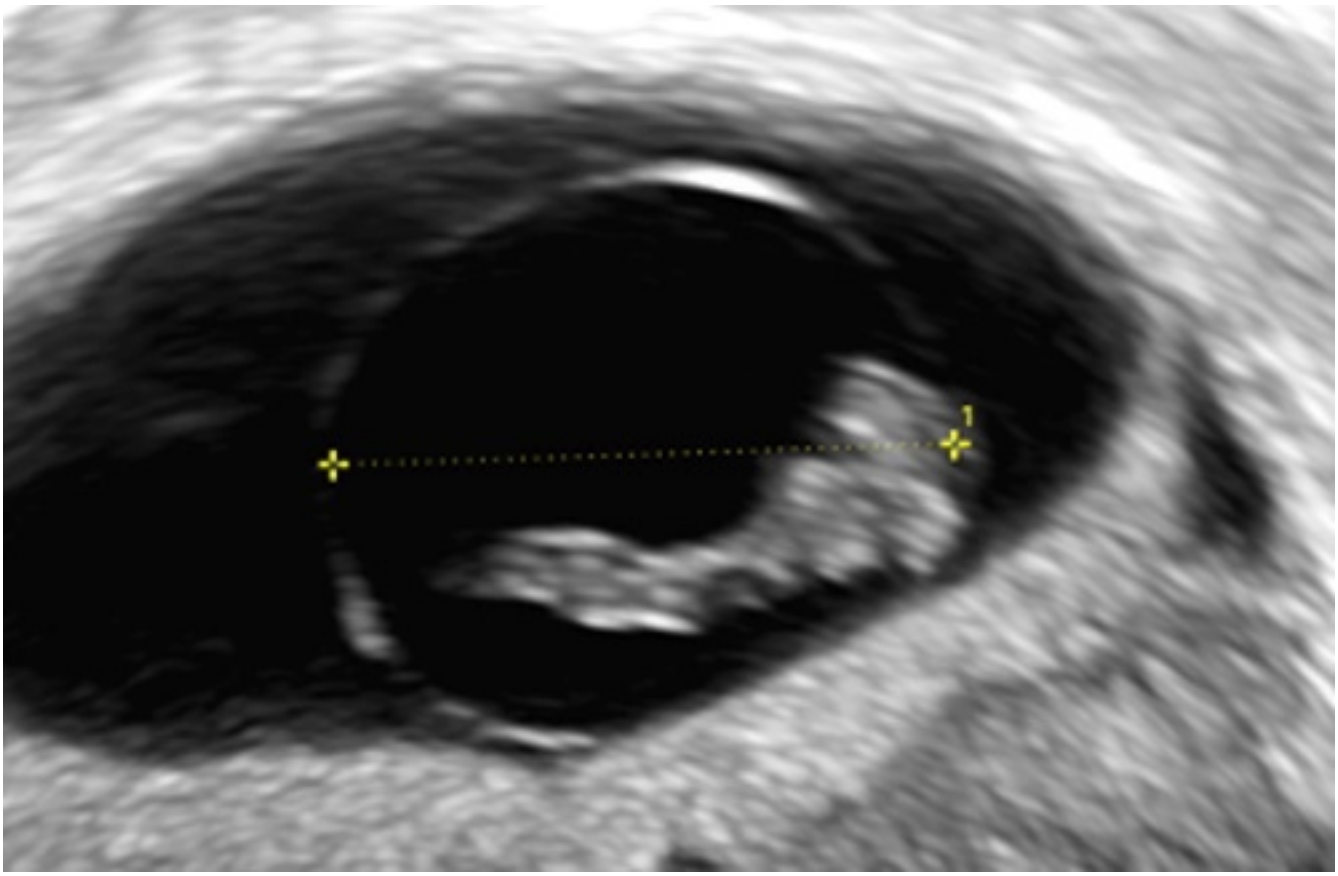
Gestational age	Predicted ASD (mm)			Predicted GSD:ASD		
(days)	5th	50th	95th	5th	50th	95th
49	3.8	7.8	11.9	2.15	2.85	3.79
50	4.7	8.7	12.7	2.04	2.71	3.59
51	5.6	9.6	13.6	1.94	2.57	3.41
52	6.6	10.6	14.6	1.85	2.45	3.24
53	7.6	11.6	15.6	1.76	2.33	3.09
54	8.6	12.6	16.6	1.68	2.22	2.94
55	9.7	13.7	17.7	1.60	2.12	2.81
56	10.8	14.8	18.8	1.53	2.03	2.68
57	11.9	15.9	19.9	1.46	1.94	2.57
58	13.1	17.1	21.1	1.40	1.86	2.46
59	14.3	18.3	22.3	1.34	1.78	2.36
60	15.6	19.6	23.6	1.29	1.71	2.27
61	16.8	20.9	24.9	1.24	1.64	2.18
62	18.2	22.2	26.2	1.19	1.58	2.10
63	19.5	23.5	27.5	1.15	1.52	2.02
64	20.9	24.9	28.9	1.11	1.47	1.95
65	22.4	26.4	30.4	1.07	1.42	1.88
66	23.8	27.8	31.9	1.04	1.37	1.82

67	25.3	29.3	33.4	1.00	1.33	1.77
68	26.8	30.9	34.9	0.97	1.29	1.71
69	28.4	32.5	36.5	0.94	1.25	1.67

ASD = amniotic sac diameter, CRL = crown-rump length, GA = gestational age, GSD = gestational sac diameter

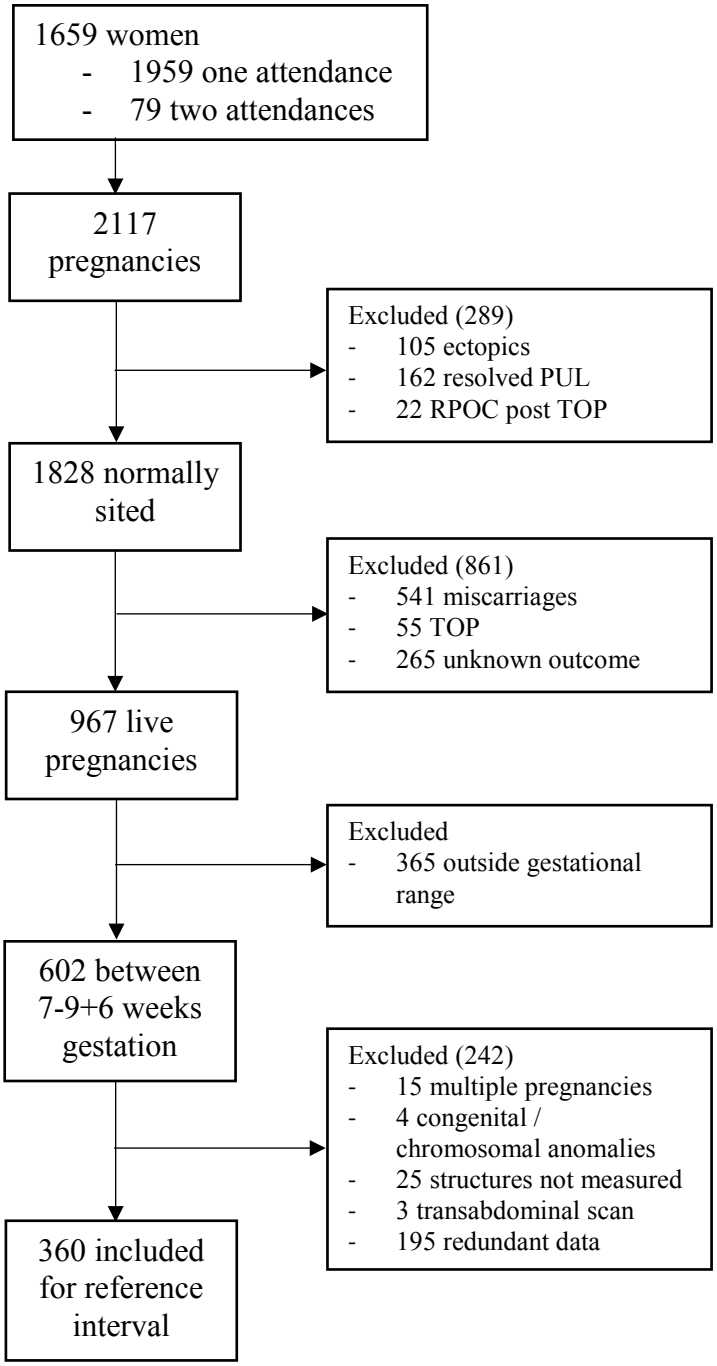


ASD Figure 1a.jpg



ASD Figure 1b.jpg

Figure 2: Study flowchart



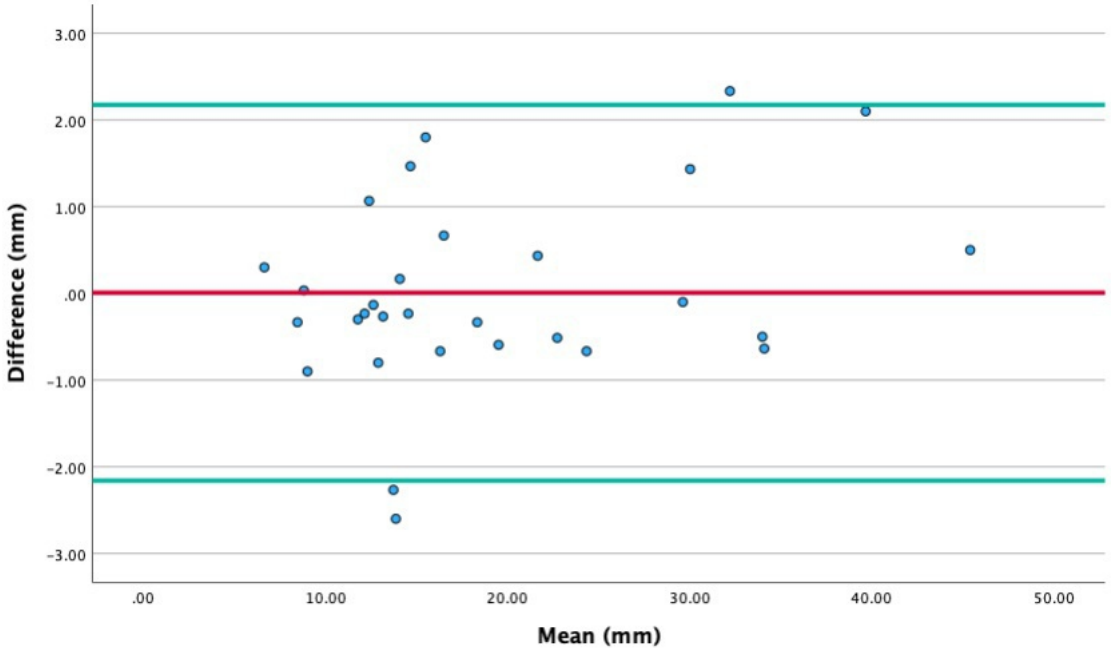


Figure 3a.jpg

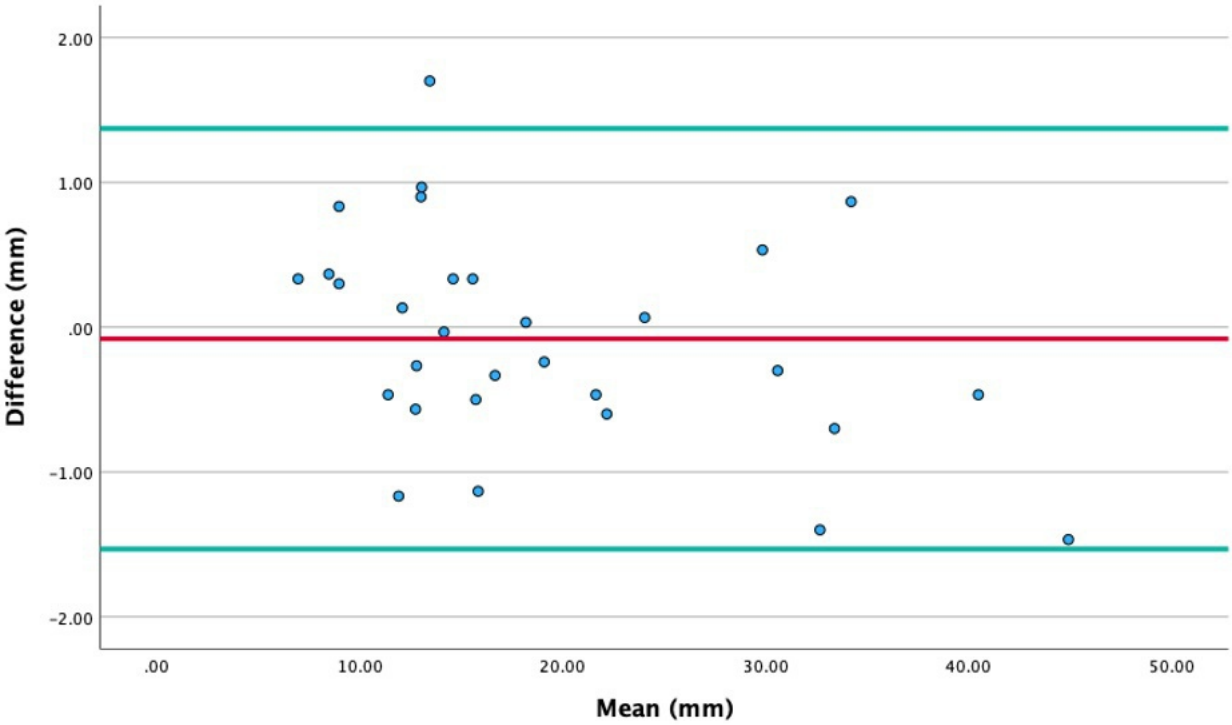
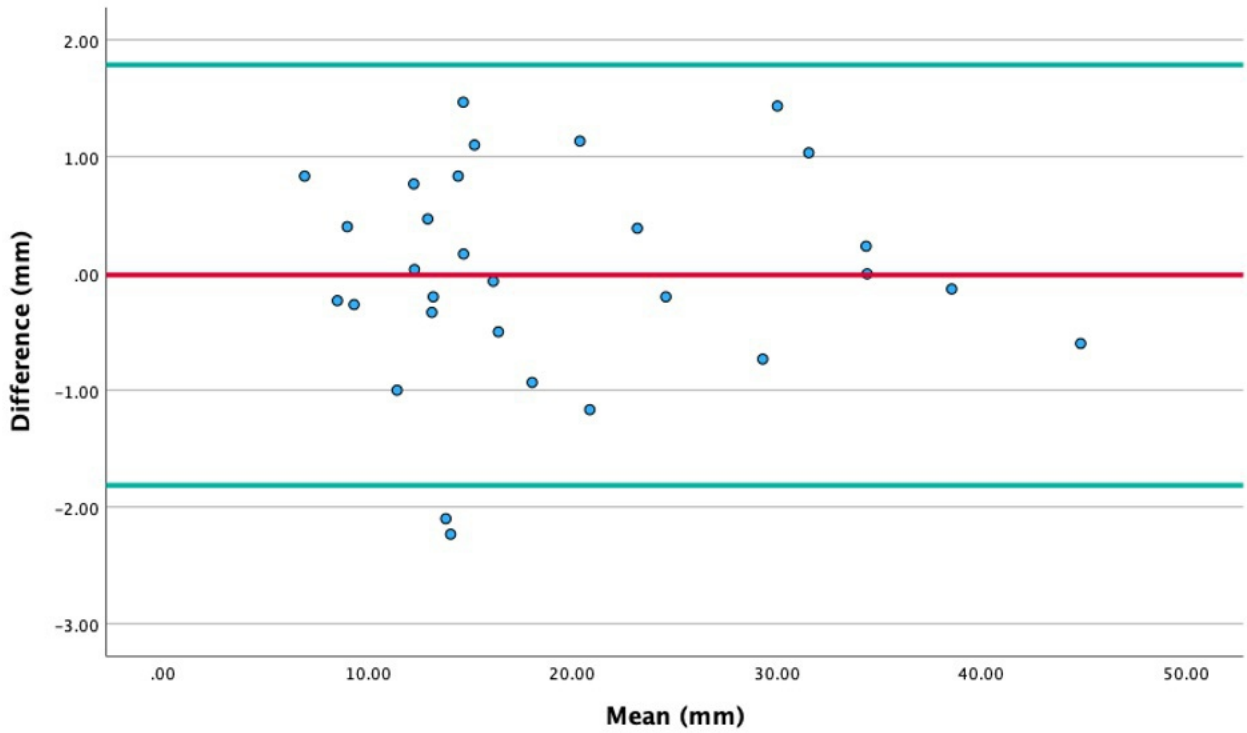


Figure 3b.jpg



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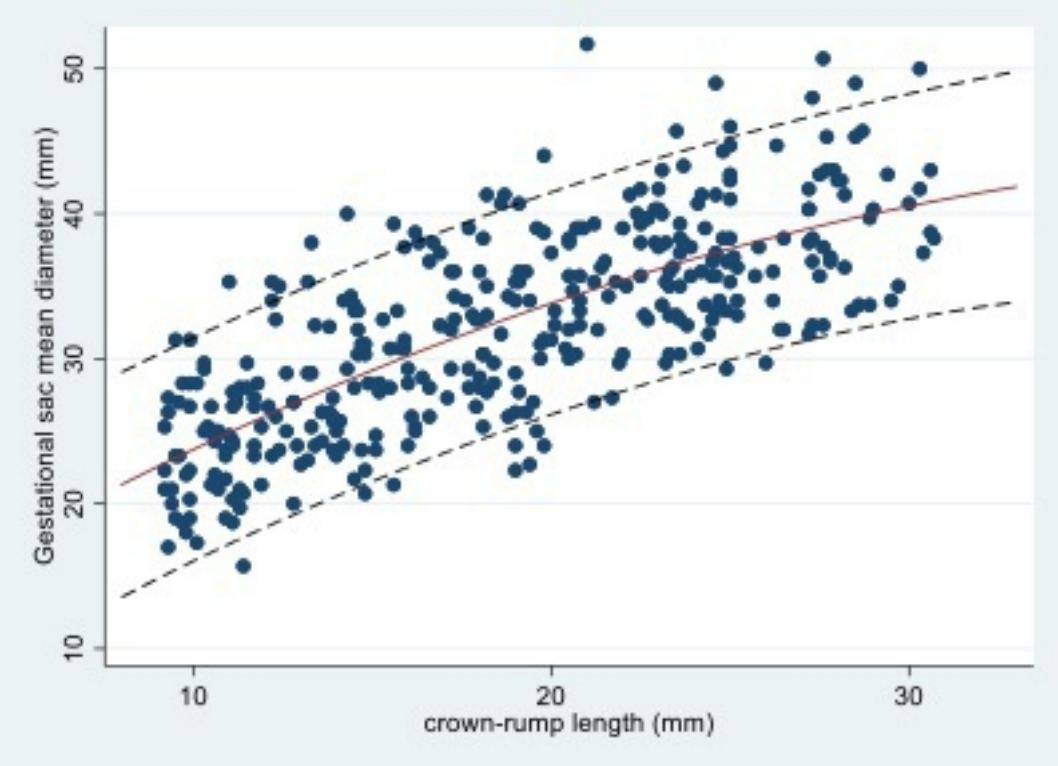

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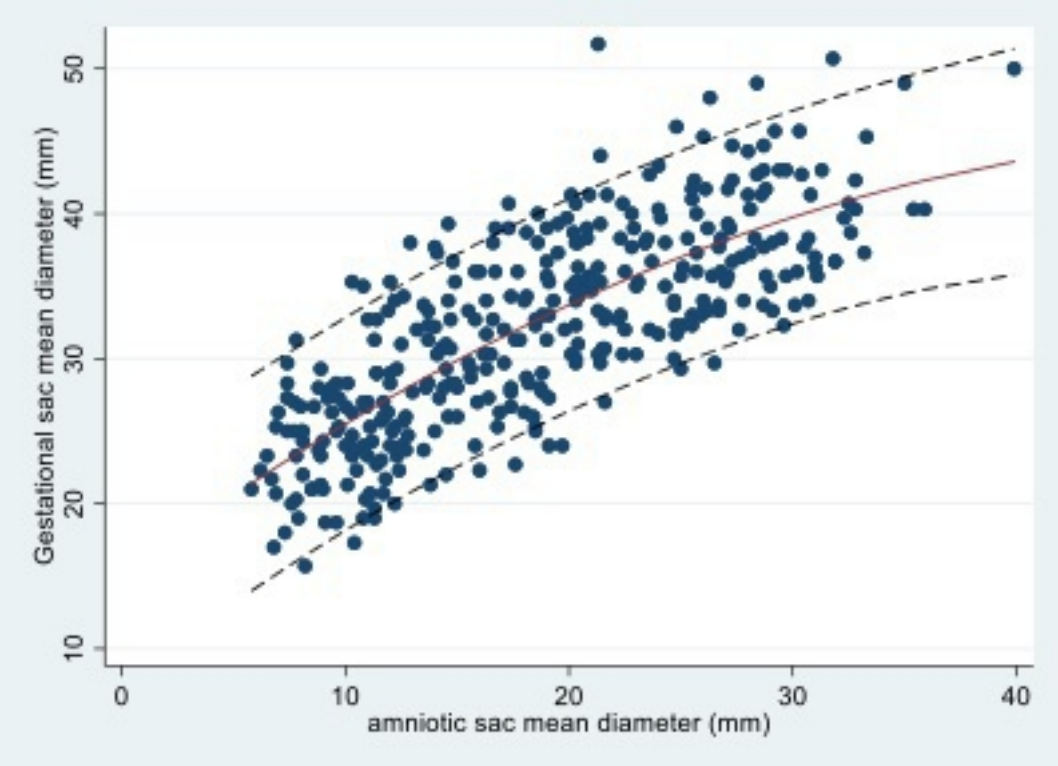

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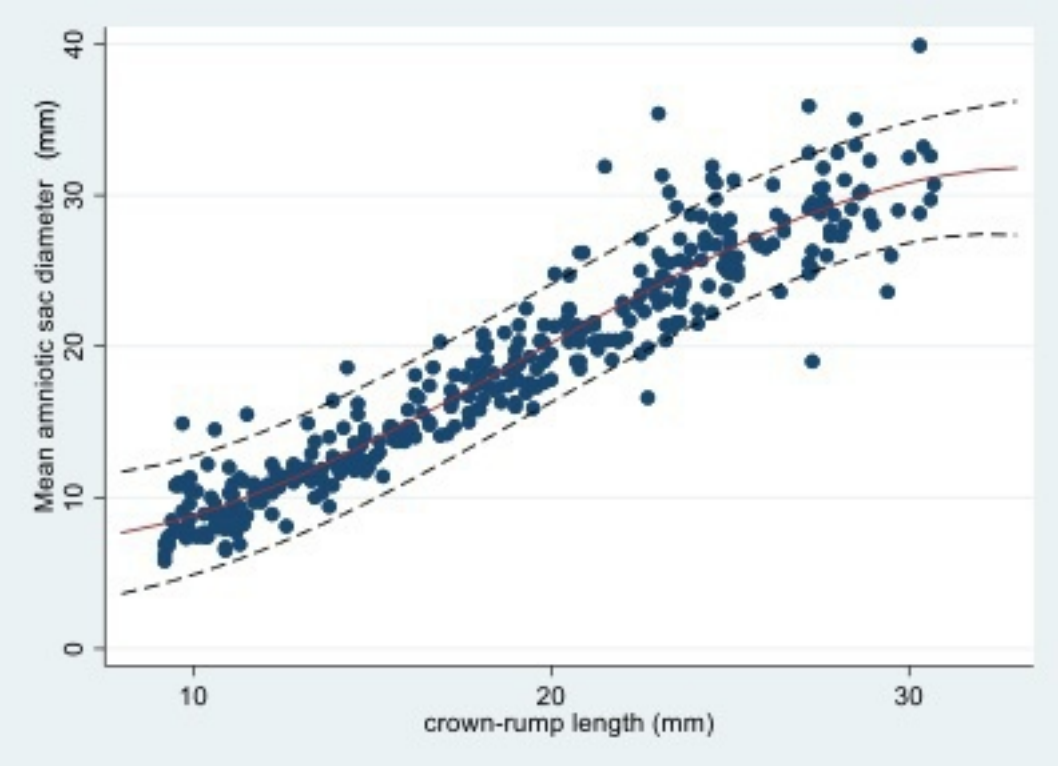

Figure 6.jpg

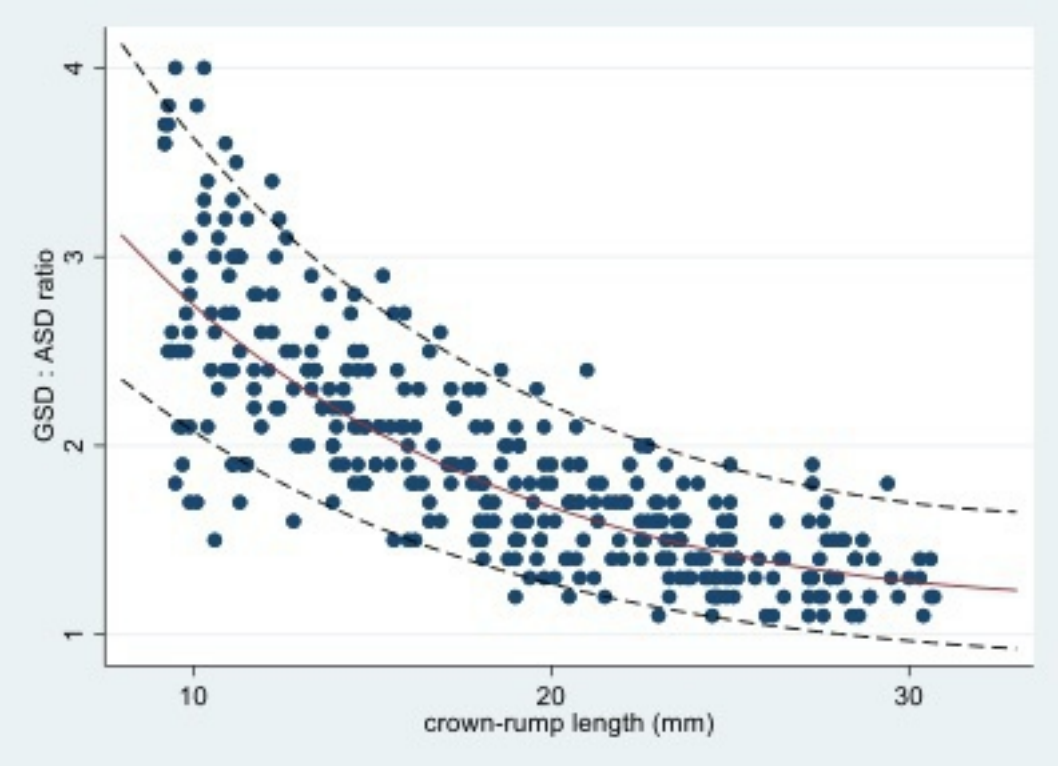

Figure 7.jpg