

Original Research Article**“Yolk sac as a predictor of pregnancy outcome – a hospital based prospective study”.**

SAMBHUNATH BANDYOPADHYAY¹, SRIJONI CHOWDHURY², SANJANA HALDER³, SASWATA BISWAS⁴, POUHALI SANYAL⁵, NIHAR RANJAN SARKAR⁶, GOURI SHANKAR KAMILYA⁷

Associate Professor, Department of Obstetrics and Gynaecology, IPGME&R, Kolkata, West Bengal, India

2.RMO cum Clinical Tutor, Department of Obstetrics and Gynaecology, Diamond Harbour Government Medical College, Diamond Harbour, West Bengal, India

3.Associate Professor, Department of Obstetrics and Gynaecology, Diamond Harbour Government Medical College, Diamond Harbour, West Bengal, India

4. Assistant Professor, Department of FMT, Medical College & Hospital, Kolkata, West Bengal, India

5. Consultant Gynaecologist & Obstetrician, Woodlands Hospital, Kolkata, West Bengal, India

6.Associate Professor, Department of Radiodiagnosis, IPGME&R, Kolkata, West Bengal, India

7.Professor, Department of Obstetrics and Gynaecology, IPGME&R, Kolkata, West Bengal, India

Corresponding Author: Dr. Srijoni Chowdhury

Article History: **submission date:** 15.11.2022 **Accepted:**05.12.2022 **Published:** 28.12.2022

ABSTRACT

The secondary yolk sac is the first extraembryonic structure that can be detected with transvaginal sonography (TVS) surrounding the chorionic cavity and can be seen from the 5th to the 12th week menstrual age, at the latest. During organogenesis and before placental circulation is established, yolk sac is the primary source of exchange between the embryo and the mother. Yolk sac has nutritive, endocrine, metabolic, immunologic, secretory, excretory and hematopoietic functions. This study was conducted to evaluate the size of secondary yolk sac as a predictor of pregnancy outcome.

In this study 200 women who presented to the antenatal OPD, between 5 and 10 weeks of gestation were evaluated with transvaginal sonography and measurements such as MSD, CRL and YSD were taken. Patients were followed up to 24 weeks of gestational age and classified as normal outcomes if pregnancy continues beyond 24 weeks and no anomalies of the foetus was noted.

There was a significant positive correlation between yolk sac diameter and crown rump length, gestational sac diameter and gestational age. A normal range of yolk sac diameter was established based on normal outcomes for each gestational week. Yolk sac diameter more than 2 standard deviations above or below the mean were considered as abnormal yolk sac diameter. Using these criteria of abnormal yolk sac diameter to predict abnormal pregnancy outcome, sensitivity was 90.85%, specificity was 55.6%, positive predictive value was 89.6% and negative predictive value was 55.2%. (P<0.001) It was also noted that sensitivity and negative predictive value were

highest when the scan was performed at 9th week and 7 th week of gestation respectively and specificity and positive predictive value was highest at 10th week of gestation. At 5th week, the results obtained weren't significant. (P=0.144).

Interpretation & Conclusion

We can conclude from the present study that measurement of the secondary yolk sac diameter between 6th to 10th week of gestation can be used as a valuable tool to predict early pregnancy outcome, even before the detection of the embryo. This in turn helps in counseling the parents regarding the risk of miscarriage and the need for follow up ultrasound examinations.

Key words: Yolk Sac Diameter; Abnormal Pregnancy Outcome; Transvaginal sonography.

INTRODUCTION

First trimester pregnancy is a dynamic period, during which changes occur in human development at a more rapid rate than any other time in human life. Understanding the normal development of early pregnancy and observing this process ultrasonographically has allowed effective monitoring of first trimester pregnancy and prediction, diagnosis and management of early signs of abnormal outcome.

About 10-20 % of all clinically recognized pregnancies end in early abortions. ^[1]The first report of ultrasound being used to evaluate first trimester loss was published in 1976. ^[2] A group of clinical factors like maternal age, race, smoking, medical comorbidities, history of vaginal bleeding were suggested for prediction of subsequent miscarriages. In addition ultrasound measurement including CRL, embryonic heart rate, yolk sac diameter, gestational sac diameter were utilized. In the first trimester yolk sac is one of the most important conceptional structure that can be assessed using ultrasound. Yolk sac size and shape has been suggested as sensitive predictor of pregnancy outcome.

Many studies on the prognostic significance of the Yolk sac for the pregnancy outcome have been performed with conventional sonography and more recently with TVS. The results are conflicting. Thus further studies on the measurement of yolk sac size and its association with normal and abnormal pregnancy outcome could help as an early predictor of pregnancy outcome and better understanding of embryos prepared for IVF –ET.

MATERIALS&METHODS

200 Pregnant women of menstrual age less than 10 week with normal BMI attending dept of obstetrics of IPGMER-SSKM Hospital (inpatients and outpatients) during the study period February 2013 –January 2014 with normal body mass index (BMI) were prospectively evaluated. All patients underwent two-dimensional transvaginal ultrasonography, which was performed by the same sonographer. We considered the following yolk sac characteristics as normal for classification: diameter: 2-5 mm; round shape; absence of degenerative changes, equal number with embryos; echogenic rim and hypoechoic center. Yolk sacs that had diameters smaller than 2 mm or larger than 5 mm; a shape that was not round (i.e., oval or distorted); the presence of degenerative changes; hyper- or hypo-echogenic rim; hyperechoic center and unequal number with embryos were considered abnormal. Based on the above

classification, patients were divided into two groups, study (abnormal yolk sac) and control (normal yolk sac).

Informed consent will be taken from the antenatal women for the performance of trans vaginal scan, which is being done routinely in the first trimester. Yolk sac diameter, crown rump length and gestational sac diameter will be measured and morphology of the yolk sac to be studied. They would be followed up till 24 wks of gestation and considered as normal pregnancy outcome if pregnancy continued beyond 24 wks and abnormal outcome if they have spontaneous abortion or demonstrable fetal anomalies. It is a hospital based prospective study of patients in the first trimester of pregnancy within 10 wks attending OPD & IPD during the study period. Exclusion Criteria:

1. Molar pregnancy
2. Women with structural anomalies of uterus and cervix
3. Women with uncontrolled endocrine disorders causing abnormal pregnancy outcome

Clinical details like age, parity, obstetric history, personal history, medical history, past history, menstrual history and details of present pregnancy in terms of period of amenorrhoea were noted at the time of scan. A detailed clinical examination including complete general physical examination and pelvic examination was done to arrive at a provisional clinical diagnosis. Ultrasonographic evaluation of patients was done using Philips HD7 machine. Transvaginal sonography was done using 5-7 mhz transducer. Head of the probe was cleaned, covered with a condom after applying ultrasonic jelly. Tip of the condom was smeared with lignocaine jelly for smooth insertion into the vagina. With the patient in the dorsal position, the probe was inserted slowly into the vagina so as to obtain a sagittal section of the uterus. The probe was rotated from 12 o'clock to 9 o'clock position (anticlockwise) to obtain a transverse section of the uterus. Yolk sac diameter, CRL and gestational sac diameter was measured. All the quantitative parameters such as yolk sac diameter, gestational age, CRL, Mean gestational sac diameter, age and parity of women etc will be described in terms of descriptive statistics such as mean and standard deviation. An abnormal yolk sac diameter was defined as being more than 2 SD above or below the mean for particular gestational age. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for abnormal yolk sac diameter in predicting abnormal pregnancy outcome. These parameters were represented graphically based on gestational age at which measurement was taken. To test for difference in Yolk sac diameter between abnormal and normal outcome Mann-Whitney U test was employed. The statistical analysis was performed using SPSS package version 20. Chi-square test was used when appropriate. Statistical significance was defined as $P < 0.05$.

RESULTS

The study was conducted in 200 antenatal women between 5 to 10 weeks of gestational age, after excluding those who met the exclusion criteria mentioned previously. Among these 13 women underwent elective termination of pregnancy and 14 cases were lost to follow up. Therefore 173 pregnancies were ultimately studied and data analyzed.

TABLE NO.1: Distribution of the study population according to pregnancy outcome

OUTCOME	FREQUENCY	PERCENTAGE
NORMAL	142	82.1
ABNORMAL	31	17.9

TOTAL	173	100
--------------	------------	------------

TABLE NO.2:PERCENTAGE OF DISTRIBUTION OF STUDY POPULATION ACCORDING TO AGE-

AGE GROUPS	FREQUENCY	PERCENTAGE	NORMAL OUTCOME	ABNORMAL OUTCOME	P Value
18-20 yrs	17	9.82	17	0	0.588
21-30 yrs	132	76.3	105	27	
31-40 yrs	24	13.8	20	4	
TOTAL	173	100	142	31	

TABLE shows 76.3% of study population were in the age group of 21-30 yrs. After performing MANN-WHITNEY U TEST in the study population the P value obtained is 0.588 and hence not statistically significant. Therefore in this study, the mean age of the mothers did not vary with the outcome.

TABLE NO.3: Distribution of gravidity of the study population

GRAVIDA	FREQUENCY	PERCENT	NORMAL OUTCOME	ABNORMAL OUTCOME	P Value
1	95	54.9	84	11	0.023
2	47	27.2	36	11	
3	41	12.1	17	4	
4	7	4.0	4	3	
5	3	1.7	1	2	
TOTAL	173	100	142	31	

The probability of abnormal outcome increases with the increase in gravidity of the patient. P value was 0.023 and hence was statistically significant

TABLE NO.4: Number of previous abortions in the study population

NO.OF ABORTIONS	FREQUENCY	PERCENTAGE	NORMAL OUTCOME	ABNORMAL OUTCOME	P Value
0	127	73.4	110	17	<0.001
1	37	21.4	29	8	
2	7	4.0			
3	2	1.2	3	6	
TOTAL	173	100	142	31	

The probability of abnormal outcome in the current pregnancy increased with increase in the number of previous abortions. The incidence of abnormal outcome was 13.4 % in patients with no history of previous abortions, while it was 66.7 % in patients with history of two or more previous abortions. P value was <0.001 and statistically significant as obtained from CHI-SQUARE TEST.

TABLE NO. 5 : TABLE BETWEEN NORMAL AND ABNORMAL YOLK SAC AND PREGNANCY OUTCOME

GA(weeks)	Normal outcome		Abnormal outcome		TOTAL
	Normal YS	Abnormal YS	Normal YS	Abnormal YS	
6	13	1	2	1	17
7	44	1	2	2	49
8	40	2	3	6	51
9	26	1	3	7	37
10	14	0	2	3	19
TOTAL	142		31		GRAND TOTAL
					173

TABLE NO.6 : YOLK SAC AS PREDICTOR OF PREGNANCY OUTCOME

GA (in weeks)	SENSITIVITY	SPECIFICITY	PPV	NPV
6	33.3%	93%	50%	86.7%
7	50%	97.7%	66.7%	95.7%
8	66.7%	95.2%	75%	93%
9	70%	96.3%	87.5%	89.7%
10	60%	100%	100%	87.5%

Statistical analysis of yolk sac diameter as a predictor of pregnancy outcome was significant when measured between 7th and 10th week of gestation ($P < 0.05$). When the measurement is taken between 40 to 42 days of gestation, the results were not significant with a P value of 0.201.

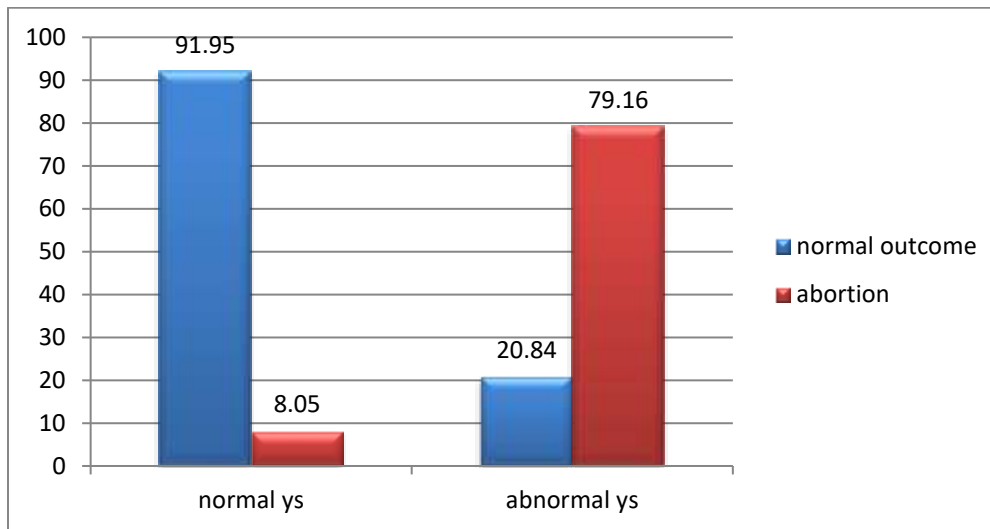
Sensitivity was highest at 9th week of gestation. NPV was highest in 7th week of gestation. Specificity and PPV was highest at 10th week of gestation.

TABLE NO.7: RELATION BETWEEN YOLK SAC SHAPE AND PREGNANCY OUTCOME

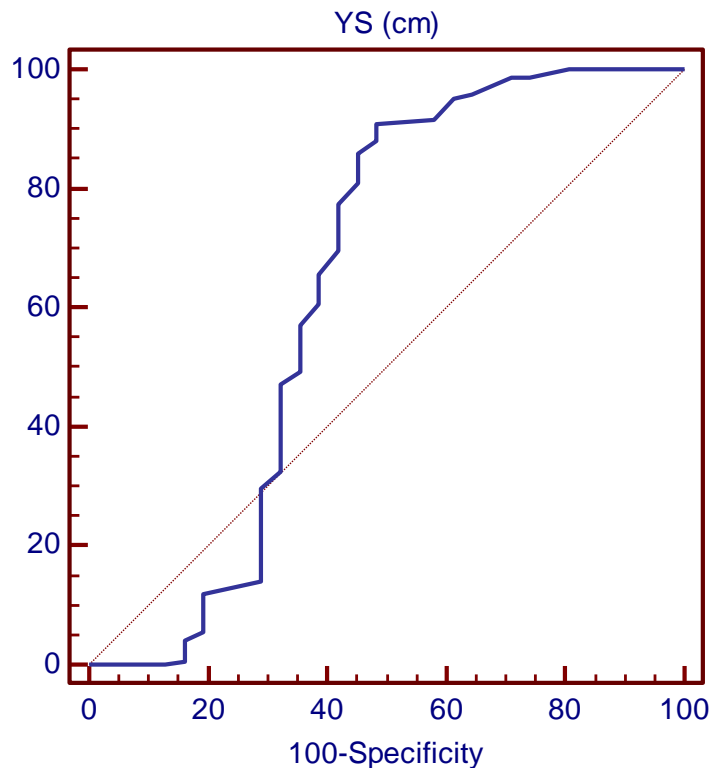
YOLK SAC SHAPE	OUTCOME						CHI-SQUARE VALUE	P-VALUE
	NORMAL		ABNORMAL		TOTAL			
	frequency	percentage	frequency	percentage	frequency	percentage		
NORMAL	137	79.2%	12	6.9%	149	86.1%	66.32	0.000
ABNORMAL	5	2.9%	19	11%	24	13.9%		
TOTAL	142	82.1%	31	17.9%	173	100%		

Since P-VALUE is significant at ≤ 0.05 level, the difference in shape of yolk sac is significant with outcome of pregnancy. Hence yolk sac shape and pregnancy outcome are associated.

GRAPH NO.1:PERCENTAGE OF ABORTIONS IN NORMAL AND ABNORMAL YOLK SACS .



GRAPH NO.2 :ROC CURVE SHOWING YOLK SAC SIZE AS PREDICTOR OF PREGNANCY OUTCOME



Youden index for the ROC Curve is 0.4246 and the cut off value is ≤ 0.55 cm. The sensitivity at 0.55cm is 90.85% and specificity is 51.6%. The positive predictive value at 0.55cm is 89.6% and negative predictive value is 55.2%.

DISCUSSION

The reliability of ultrasonographic visualization of the human yolk sac is well established^[3]. The mean age of the study population was 26 (25.97) years and 76.3 % of the study population belonged to the age group of 21- 30 years. In this study 54.9% of the study population were primigravidae.

INCIDENCE OF ABNORMAL PREGNANCY OUTCOME:

In the present study we had 17.9 % incidence of abnormal pregnancy outcome. This is in concordance with the study by Roth et al^[4] who estimated the frequency of spontaneous abortion to be 15% of recognizable pregnancies. In the study conducted by Lindsay et al^[5] to evaluate the role of yolk sac size and shape as predictor of pregnancy outcome, the incidence of abnormal pregnancy outcome was 32.7%(159/486). In another study by Sanam Moradan et al^[10] 10.7 % (20/191) had abnormal outcome. But the criteria for defining an abnormal outcome varies in different studies, for example in our study, abnormal outcome was defined as spontaneous abortion, or missed abortion before 24 weeks of gestation or demonstrable foetal anomalies whereas, Lindsay et al considered abnormal outcome as first trimester embryonic or fetal death or demonstrable fetal anomaly. In an Indian study Nawal Rajani et al^[6] the incidence of abnormal outcome was 20% which included missed abortion and blighted ova before 12 weeks of gestation. In our study, the probability of abnormal pregnancy outcome did not vary with maternal

age. ($P = 0.588$). This is contradictory to the results of previous studies which show an increase in the incidence of miscarriage with increase in maternal age^[7,8,9]. According to these studies, until age 30, the incidence of miscarriage is approximately 12%, thereafter the rate increases rapidly, exceeding 50% in the women older than 45 years.^[9]

Our study indicates that the probability of abnormal outcome increases with the increase in gravidity of the patient ($P = 0.023$), with significant increase in the risk of abortion with Gravida 4 and more (42.9% to 66.7%). Also the present study shows that history of previous abortion increases the risk of

abnormal outcome in the subsequent pregnancies ($P < 0.001$). With no previous abortion the risk of abnormal outcome is 13.4%, which increases to 21.6% with history of 1 previous abortion and 66.7% with 2 or more previous abortions. This risk is more than that found in a study by Regan et al^[10] in which, for patients with a history of RPL, the risk of subsequent pregnancy loss is estimated to be 24% after two clinically recognized losses, 30% after three losses, and 40% to 50% after four losses.

CORRELATION OF YOLK SAC DIAMETER WITH GESTATIONAL AGE, CROWN RUMP LENGTH AND MEAN GESTATIONAL SAC DIAMETER IN PREGNANCIES WITH NORMAL OUTCOME:

In this study, a highly significant positive correlation was found between yolk sac diameter and crown rump length (spearman rho coefficient = 0.446), gestational sac diameter (spearman rho coefficient = 0.488) and gestational age (spearman rho coefficient = 0.496). [$P < 0.001$] This was similar to the results obtained in a study by Nawal Rajani et al. in IMS BHU, Varanasi, Uttar Pradesh^[6]. In their study they found a highly significant positive correlation between mean YSD and gestational age ($r = 0.74$) and mean YSD and mean gestational sac diameter ($r = 0.739$) with p value < 0.001 and a significant correlation was detected between mean YSD and CRL ($r = 0.355$, $P < 0.05$) Kucuk et al^[11] found a highly significant positive correlation between yolk sac diameter and menstrual age, yolk sac and crown rump length and between yolk sac and mean gestational sac diameter as $r = 0.9581$ ($p < 0.001$), $r = 0.9427$ ($p < 0.0001$) and $r = 0.8855$ ($p < 0.001$) respectively.

BIOMETRY OR GROWTH OF YOLK SAC WITH GESTATIONAL AGE IN PREGNANCIES WITH NORMAL OUTCOME:

In our study, progressively increasing mean YSD was found with advancing gestational age between 6th and 9th week of gestation, from 0.365 cm to 0.504 cm following which it starts decreasing in size by 10th week.

Cepni et al^[12] demonstrated the steady increase in YSD from 5 to 11 weeks of gestation in normal pregnancies after which it disappears by 12 weeks. Chama et al^[13] reported a linear increment in mean YSD from 2.27 mm at 5 weeks of gestation to 5.61 mm at 11 weeks of gestation. Lindsay et al^[14] reported that yolk sac grows at a rate of approximately 0.1 mm per mm growth of the MSD when the MSD is less than 15 mm and then slows to 0.03 mm per mm growth of MSD

In the present study Mann Whitney u test was applied to test the significance of difference between the means of YSD of normal and abnormal pregnancy outcome. The difference between

the mean yolk sac diameters between normal and abnormal outcomes was not significant except at 8th wk with a P value of 0.005. As depicted in table 11, 12 and 14 and Graph 12, the yolk sac diameter of abnormal outcomes varied widely, both above and below the mean yolk sac diameter for normal outcomes, hence the mean yolk sac diameter of abnormal outcomes may not differ significantly from that of normal outcomes. This is contradictory to the study by NawalRajani et al^[15] in which a highly significant difference was detected between mean YSD of both groups (P<0.001).

VALUE OF ABNORMAL YOLK SAC DIAMETER AS A PREDICTOR FOR ABNORMAL PREGNANCY OUTCOME

In this study a range of yolk sac diameter with 95% confidence limits was established based on the pregnancies with normal outcomes for each gestational week. With respect to this range, there were 24 pregnancies with abnormal yolk sac diameter, with 9 of them measuring lesser than 2SD below the mean and 15 measuring more than 2 SD above the mean. 19 of these pregnancies subsequently had an abnormal outcome. When both the groups are combined, the sensitivity of predicting an abnormal outcome was 90.85 %, specificity was 51.6 % and PPV was 89.6% (P<0.001) in comparison to 26.9%, 92.7% and 51.1% respectively, reported by Lindsay et al^[14]. The highest sensitivity of predicting an abnormal outcome was 70 % at 9 wks, specificity was 100 % and PPV was 100% at 10 wks. The results of our study are more likely comparable to the results of the study conducted by Kucuk et al^[16] They found a yolk sac diameter out of 2 standard deviations of the mean for the menstrual age allowed prediction of an abnormal pregnancy outcome with a sensitivity of 65%, a specificity of 97%, a PPV of 71% and a NPV of 95%, whereas in our study we found 90.85 %, 51.6 %, 89.6% and 55.2% respectively.

In our study we have also estimated the ideal time to perform the transvaginal scan, in order to achieve the highest possible sensitivity, specificity, positive and negative predictive value. Sensitivity was highest at 9th week of gestation. Specificity and PPV was highest at 10th week of gestation. NPV was highest at 7th wk. Statistical analysis of yolk sac diameter as a predictor of pregnancy outcome was significant when measured between 7th and 10th week of gestation, i.e. from 43 to 70 days of gestation (P < 0.05). When the measurement were taken between 40 to 42 days of gestation, the results were not significant with a P value of 0.144. This is in contrast to the studies done previously in which yolk sac could be accurately evaluated from 5 completed weeks of GA^[6,14,17]. This might have resulted due to the small yolk sac size at this time of gestation combined with various other confounding factors like inaccuracy of menstrual dating, expertise of the sonographer, resolution of the transducers.

In a more recent study by Berdahl, D. M., J. Blaine, et al^[18], the authors have examined the value of a specific cut-off (5 mm) to allow easier patient counselling. They concluded pregnancies with MSD \geq 5 mm on early ultrasound require monitoring and counselling about a threefold increased risk for first-trimester loss independent of maternal risk factors such as age, body mass index, polycystic ovary syndrome, smoking, and diabetes. In addition, their study shows for the first time that enlarged yolk sac diameter may be associated with an increased risk of preterm delivery. Likewise in our study also we calculated the cut-off value of YSD and found it to be 0.55 cm.

In total 86.1% of reviewed pregnancies (149 of 173) had yolk sacs with regular shape and 13.9% (24 of 173) had irregular shape. An abnormal yolk sac shape was detected in 19 cases among the 31 aborted pregnancies (11%). Comparison performed by Chi-square test revealed statistical significance among the rates of abortion in pregnancies with normal and abnormal yolk sac shape ($p < 0.000$). A previously conducted study by Lindsay et al^[81] with 486 pregnancies of less than 10 wks found 7 cases with abnormal shape but with normal size (1.4%). Of them 4 continued till term (3 healthy babies, 1 had omphalocele), 2 had spontaneous abortion and 1 had embryonic death. Another similar study by Kucuk et al yolk sac shape was abnormal in 4.5% of normal pregnancies (10 of 219) and 29% of spontaneously aborted (9 of 31) pregnancies in first trimester. Both the studies claimed persistence of abnormal yolk sac shape might be useful predictor of abnormal pregnancy outcome.

While determining abortion rate among two groups - we found rate was much higher (79.16%) in the group having abnormal yolk sac size and shape than the other group with normal parameters. This finding is supported by another study by StamponC et al.

CONCLUSION

We can conclude from the present study that measurement of the secondary yolk sac diameter between 6th to 10th week of gestation can be used as a valuable tool to predict early pregnancy outcome. As the yolk sac is the first structure to appear in the gestational sac, confirming an intra uterine pregnancy, using yolk sac measurement as a tool to evaluate pregnancy outcome, provides a mode of early prediction of pregnancy outcome, even before the detection of the embryo. This in turn helps in counselling the parents regarding the risk of miscarriage and the need for follow up ultrasound examinations. It is particularly helpful to counsel patients with history of threatened or recurrent abortion who abort despite resting in the hospital for many weeks. It also helps in pursuing a more active line of management if required. Based on the results of this study and data available from the literature, it is certain that abnormal yolk sac diameter is associated with poor pregnancy outcome. But there is uncertainty regarding the causality of the poor pregnancy outcome associated with abnormal yolk sac size. Very few authors suggest association of chromosomal abnormalities with abnormal yolk sac size, some negate this association. Some suggest association of endocrine abnormalities such as Type 1 diabetes mellitus with enlarged yolk sac. Further studies are required to establish causality of poor pregnancy outcome with abnormal yolk sac size, so that a line of management can be planned for such patients, which may include karyotyping of the couple, karyotyping of the abortus, investigations for detecting any possible endocrine factors associated with such outcome and to plan further line of treatment of such patient.

ACKNOWLEDGEMENT

It is a great pleasure to express my heartiest gratitude and my respect to my teacher Dr S.N Bandyopadhyay ,Dr G.S Kamilya for his constant support, encouragement, guidance, supervision and kindhelp with constructive criticism during whole course of my work.

I am very grateful to all my subjects who consented and participated in my study whose constant inspiration, active support and help made possible for me to complete this thesis on time.

I also express my heartiest gratitude to Dr.NiharRanjanSarkar,Asso prof IPGMER –SSKM hosp who carried all measurements without the constant support of whom this thesis could have never reached its birth.

REFERENCES

1. Dutta D.Textbook of obstetrics. 7th ed. Konar H, editor. Kolkata: New Central Book Agency (P) Ltd; 2011.
2. Turner MJ, Flannelly GM, Wingfield M, et al. *The miscarriage clinic: An audit of the first year.* Br J ObstetGynaecol. 1976; 306-08.
3. Mantoni M. Pedersen J.F. *Ultrasound visualization of the human yolk sac.* J Clin Ultrasound. 1979; 7(6): p. 459-60.
4. Roth, D.B. *The frequency of spontaneous abortion.* Int J Fertil. 1963; 8: p.
5. Lindsay D.J.et al. *Yolk sac diameter and shape at endovaginal US: predictors of pregnancy outcome in the first trimester.* Radiology. 1992; 183(1): p. 115-8.
6. NawalRajani, K.S., Jain Deepika, Khuteta Rakesh P, Meena Vinay K, *To Assess Value of yolk sac in predicting pregnancy outcome during first trimester: Observational study.* NATIONAL JOURNAL OF MEDICALRESEARCH. 2012; 2(3): p. 343-4.
7. Bennett G.L. et al. *Subchorionic hemorrhage in first-trimester pregnancies:prediction of pregnancy outcome with sonography.* Radiology, 1996. 200(3):p. 803-6.
8. Makrydimas, G., et al., *Fetal loss following ultrasound diagnosis of a live fetus at 6-10 weeks of gestation.* Ultrasound in Obstetrics & Gynecology. 2003; 22(4): p. 368-72.
9. Gindoff, P.R., R. Jewelewicz. *Reproductive potential in the older woman.* FertilSteril, 1986. 46(6): p. 989-1001.
- 10.Regan, L., P.R. Braude, and P.L. Trembath, *Influence of past reproductive performance on risk of spontaneous abortion.* BMJ, 1989; 299(6698): p. 541-5.
11. Kucuk, T., et al. *Yolk sac size and shape as predictors of poor pregnancy outcome.* Journal of Perinatal Medicine, 1999; 27(4): p. 316-20.
12. outcomeIsmail Cepni, Tugan Bese, Pelin Ocal, Erdal Budak, Mehmet Idil, Professor M. Feridun AksuSignificance of yolk sac measurements with vaginal sonography in the first trimester in the prediction of pregnancy First published: 31 December 2010<https://doi.org/10.3109/00016349709034911>
14. Lindsay, D.J. et al. *Yolk sac diameter and shape at endovaginal US:predictors of pregnancy outcome in the first trimester.* Radiology, 1992; 183(1): p. 115-8.
15. Levi, C.S., E.A. Lyons, and D.J. Lindsay. *Early diagnosis of nonviable pregnancy with endovaginal US.* Radiology, 1988; 167(2): p. 383-5.

16. Kucuk, T., et al. *Yolk sac size and shape as predictors of poor pregnancy outcome*. Journal of Perinatal Medicine. 1999; **27**(4): p. 316-20.
17. Stampone, C. et al. *Transvaginal sonography of the yolk sac in normal and abnormal pregnancy*. J Clin Ultrasound. 1996; **24**(1): p. 3-9.
18. Berdahl, D.M., et al. *Detection of enlarged yolk sac on early ultrasound is associated with adverse pregnancy outcomes*. FertilSteril. 2010; **94**(4): p.1535-7.
19. Chama CM, Marupa JY and Obed JY. *The value of the secondary yolk sac in predicting pregnancy outcome*. J ObstetGynaecol. 2005; **25**(3): p. 245-7.
20. Reece E.A., et al. *Ultrastructural analysis of malformations of the embryonic neural axis induced by in vitro hyperglycemic conditions*. Teratology, 1985;**32**(3): p. 363-73.