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Research Article

Evaluation of Yolk Sac Diameter and Embryonic Heart Rate as Prognostic Factors of Gestational Outcome in Early Singleton Pregnancies

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Abstract: It has been previously suggested that abnormal yolk sac characteristics and slow embryonic foetal heart rate are associated with poor gestational outcome and these parameters can be used to assess foetal viability in first trimester of pregnancies. However there is paucity of evidence in Indian medical literature. The purpose of present study was to examine the role of yolk sac characteristics and early embryonic heart rate (EHR) between 6 to 9 weeks of pregnancies and to study their associations with spontaneous abortions. This prospective observational study included 280 low risk healthy singleton pregnant women attending antenatal clinic in a tertiary medical institution in early gestation. Transvaginal ultrasound was carried out to quantify yolk sac morphometry and using M mode, early embryonic heart rate was measured. 90% (252/280) of subjects satisfied normal yolk sac morphometry according to Nyberg criteria and 99.2% of them (250/252) had ongoing pregnancies. 28 patients had abnormally appearing yolk sac and 34.5% of them (10/28) subsequently aborted. Similarly 99.3% (266/268) who had normal embryonic heart rate (≥100 bpm) had successful outcome. Abnormal heart rate (<100 bpm) was detected in 12 pregnancies and majority of them (10/12, 83.3%) ended up in foetal demise. Yolk sac diameter between 2-5 mm showed sensitivity, specificity and accuracy of 93.3%, 83.3% and 92.9% for successful gestational outcome. The respective figures for embryonic heart rate (≥ 100 bpm) were 99.3%, 83.3% and 98.5%. The embryos with good yolk sac parameters and normal heart rate have high potential for survival. The information obtained by first trimester vaginal ultrasound may be used to prognosticate pregnancies that are complicated by previous bad obstetric history.
Keywords: Yolk sac, Embryonic Heart Rate (EHR), First trimester, Spontaneous abortions.

INTRODUCTION

It is estimated that approximately 30-40 % of human pregnancies result in spontaneous abortion during the first trimester after implantation. Significant number of losses predominantly occurs very early in gestation, but once the embryonic heart activity appears the rate of spontaneous abortion gradually decreases to 2-5 % [1]. However intrauterine gestational sac is the first one to appear sonographically, followed by the yolk sac and the foetal pole with cardiac activity [2]. Within the gestational sac, yolk sac is the first evident embryonic structure [3]. It is usually visible between the fifth and twelfth week of pregnancy as a round anechoic area; after which it undergoes degeneration [4]. Recent studies have investigated the size, structure and function of the yolk sac in addition to embryonic heart rate in evaluation and prognosis of first trimester pregnancy loss. By transvaginal sonogram, we can accurately demonstrate EHR (Embryonic Heart Rate) and assess the pregnancy outcome in those with bradycardia [5]. The embryonic heart beat can usually be identified at prenatal ultrasonography by 6 weeks gestation in M-mode [6]. Several studies have documented that a slow embryonic heart rate at 6.0–7.0 weeks gestation is associated with a high rate of first-trimester foetal demise, and the demise often occurs soon after the slow heart beat is detected [7, 8]. The purpose of the present study was to examine whether yolk sac diameter and early embryonic heart rate (EHR) could serve as prognostic factors in evaluating pregnancy outcome.

MATERIALS AND METHODS

This prospective study was conducted in a tertiary medical college hospital in South Karnataka from 2010 to 2012.The subjects included 280 pregnant women between 6-9 weeks of gestation who were attending the antenatal clinic in Department of Obstetrics and Gynaecology. Institutional ethical committee and hospital authorities gave necessary

clearance to conduct the study. An implied consent was obtained from all women who were participating in the study. Exclusion criteria were pregnancies complicated by blighted ovum, previous threatened abortions, previous miscarriages and absent embryonic cardiac activity. All pregnancies had an ultrasound evaluation using Philips HD11XE ultrasound equipment with a capacity of simultaneous B-mode and M-mode scanning. Various ultrasound parameters such as gestational sac diameter, crown- rump length, cardiac activity, yolk sac diameter, choriodecidual reaction and embryonic heart rate were studied. Gestational age was determined by ultrasonographic measurements of foetal crown-rump length (CRL) and correlated with last menstrual period (LMP).The yolk sac diameter (YSD) was determined by placing the calipers on the inner limits of the longer diameter (Fig. 1). Embryonic heart rate (EHR) measurements were obtained transvaginally using M-mode sonography (Fig. 2). All pregnancies were followed for survival till completion of 20 weeks by either a subsequent ultrasound scan or a telephone interview. Abnormal outcome was defined as abortion less than 20 weeks and normal outcome as continuation of pregnancy beyond 20 weeks or live birth subsequently.

Fig. 1: Intrauterine gestational sac with 5 mm yolk sac

Fig. 2: Measurement of embryonic heart rate in M mode

Sample size estimation

Doubilet *et al*. studied 1425 singleton pregnancies between 6.3 to 7.0 weeks and determined the mean heart rate (± SD) as 125.3 ± 15.9 bpm (beats per minute)[9]. They found that heart rate was <100 bpm in 61 patients (4.28%). Based on this information we calculated required sample size so that we could achieve 90% power and 0.05 significance level, by using the formula,

$$n = \frac{(z\alpha + z\beta)^2\sigma^2}{p\Delta\mu^2}$$

Where, $z\alpha = 1.96$ (critical value that divides the central 95% of z distribution from 5% in the tails),

$z\beta = 1.28$ (critical value that separates the lower 10% of distribution from upper 90%),

$\sigma = 15.9$ (standard deviation), $\Delta\mu = 25.3$ (required difference in mean for significant difference i.e., 125.3-100),

p = prevalence of slow embryonic heart rate (4.28% or 0.0428)

Accordingly, we needed 97 cases. However in the study period it was possible to recruit 280 patients in the study period. Thus the number of subjects in our study is more than adequate for statistically significant observations.

SPSS version 16 for windows (SPSS Inc., Chicago, IL, USA) was used for analysis of data. Descriptive analysis was done to find the mean and standard deviation. Analysis of variance (ANOVA) was performed to compare various groups. A "p" value of 0.005 or less was considered as statistically significant. Diagnostic ability of two different embryonic parameters (yolk sac and embryonic heart rate) were analysed for various test statistics such as sensitivity, specificity, positive predictive value, negative predictive value, likelihood and odd's ratio etc.

RESULTS

Total of 280 patients were enrolled in this study. Of these 268 (95.7 %) had normal pregnancy outcome and 12 (4.3%) patients had abnormal outcome. The study population included 206 primigravidae and 74 multigravidae. There were 6 abortions in each group. The percentage statistics along with significance levels are given in Table 1.

We wanted to know whether the rates of abortions were influenced by maternal age. Table 2 shows detailed analysis of age factor in different groups (Mean ± SD). However comparison of means in different outcomes and parity status indicated that there were no significant differences with regard to maternal age in any of these groups.

Table 1. Effect of Gravidity on pregnancy outcome

Gravidity	No. of Cases	Pregnancy outcome				Statistical Inference
		Abortions		Ongoing pregnancy		
		No.	%	No.	%	
Primigravidae	206	6	2.91	200	97.09	Chi-square = 3.583 (df = 1, p = 0.0584), Not Significant
Multigravidae	74	6	8.11	68	91.89	

Table 2: Influence of maternal age on pregnancy outcome

Gravidity	No. of Cases	Pregnancy outcome				p Value
		Abortions		Ongoing pregnancy		
		No.	Mean ± SD	No.	Mean ± SD	
Primigravidae	206	6	27.34 ± 2.45	200	27.4 ± 3.47	0.97 (Not Significant)
Multigravidae	74	6	27.76 ± 2.04	68	27.12 ± 3.46	0.66 (Not Significant)

Table 3 shows pregnancy outcome depending upon the appearance of yolk sac. We used Nyberg criteria to decide the appearance of yolk sac [10]. The major criteriae considered were a large sac (≥ 25 mm mean sac diameter without an embryo or ≥20 mm mean sac diameter without a yolk sac) and distorted shape of the gestational sac. The minor criteriae included thin decidual reactionless than 2 mm, irregular contour of gestational sac, absent double decidual sign, week decidual amplitude and low position of the sac. We considered the findings as abnormal if any one major criteria or any three minor criteriae were present. Accordingly 252 patients had normal appearance and of them 99.2% (250/252) experienced live pregnancy.

Whereas those with abnormal findings (n=28), only 65.5% had ongoing pregnancy and 34.5% (10/28) aborted. These findings too were statistical significant.

Table 3: Outcome of pregnancy based on appearance of yolk sac

Appearance of yolk sac	No. of Cases	Pregnancy				p Value (Significance)
		Abortions		Survived		
		No.	%	No.	%	
Normal	252	2	0.8	250	99.2	0.001 (Highly Significant)
Abnormal	28	10	34.5	18	65.5	

Table 4 analyses the pregnancy outcome depending upon size of yolk sac. The pregnancy outcome was optimum when the sac diameter ranged between 2 to 5 mm. The live pregnancy rate increased to 99.2% (250/252) with this yolk sac diameter range. However when the yolk sac diameter fell outside of this range live pregnancy rates were significantly decreased (for < 2 mm - 50%, > 5 mm – 66.6%).

Table 4: Outcome of pregnancy based on size of yolk sac

Size in mm	No. of Cases	Pregnancy				p Value (Significance)
		Abortions		Survived		
		No.	%	No.	%	
< 2	4	2	50	2	50	0.001 (Highly Significant)
2 - 5	252	2	0.8	250	99.2	
>5	24	8	33.4	16	66.6	

We further divided the total cases in to two groups based on the embryonic heart rate < 100 (Abnormal) or ≥ 100 beats per minute (Normal). Ten abortions occurred when heart rate was abnormal (10/12, 83.3%), whereas, those with normal embryonic heart rate only 2 patients (2/268, 0.7%) had abortion. These observations were highly significant. Table 5 describes abortion and live pregnancy rates according to embryonic heart rate. In Two patients who had normal embryonic heart rate experienced pregnancy loss and one of these abortions had cervical incompetency.

Table 5: Outcome of pregnancy based on embryonic heart rate (HER)

Embryonic Heart Rate (bpm)	No. of Cases	Pregnancy				p Value (Significance)
		Abortions		Survived		
		No.	%	No.	%	
Normal (≥100)	268	2	0.7	266	99.3	0.0001 (Highly Significant)
Abnormal (<100)	12	10	83.3	2	16.7	

Table 6 describes diagnostic abilities of two ultrasound markers of foetal viability (normal embryonic heart rate and yolk sac) individually. Both parameters have comparable test performances, except that specificity for abnormal yolk sac parameters is poor, indicating that when yolk sac diameter falls out of the range, the pregnancy need not always be compromised. The presence of normal cardiac activity in the embryo ensures good viability and a very good chance of continuation of pregnancy.

Table 6. Diagnostic test parameters for normal embryonic heart rate and yolk sac diameters

Test Parameters	Yolk Sac (2 – 5 mm)	Embryonic Heart Rate (≥100 bpm)
Sensitivity	93.3% (90.3% - 96.3%)	99.3% (98.2% - 100.0%)
Specificity	83.3% (62.2% - 100.0%)	83.3% (62.2% - 100.0%)
Positive Predictive Value	99.2% (98.1% - 100.0%)	99.3% (98.2% - 100.0%)
Negative Predictive Value	35.7% (18.0% - 53.5%)	83.3% (62.2% - 100.0%)
Likelihood Ratio for +ve Test	5.60 (1.58–19.84)	5.96 (1.68 - 21.1)
Likelihood Ratio for -ve Test	0.08 (0.05 - 0.13)	0.009 (0.002 - 0.04)
Accuracy	92.9% (89.8% - 95.9%)	98.5% (97.2% - 99.9%)
False positive rate	16.6%	16.7%
False negative rate	6.7%	0.8%
Odd's ratio	69.4	665
(figures in bracket indicate 95% confidence interval)		

DISCUSSION

Before placenta is fully formed, the developing embryo derives its nutrition from yolk sac. The yolk sac reaches its highest level of functional activity between 4th and 7th week of gestation and meets metabolic, endocrine, immunologic and haemopoietic needs of embryo in early stages of its development [11]. The appearance of yolk sac is a marker of successfully growing gestational sac and is identified by transvaginal ultrasound between 4th and 5th week of gestation prior to appearance of foetal pole and embryonic heart [12]. It is a circular structure and identified by its thin echogenic rim and central hallow. The initial diameter is around 3 - 4 mm and gradually it increases at the rate of 0.1 mm per day and finally undergoes atresia by 10th to 11th week of pregnancy [13]. Once the placental circulation is established, the blood supply to yolk sac gradually decreases and finally disappears. If a large yolk sac persists, it indicates aberrant embryonic development and high chance of miscarriage [14]. Other variations in appearance of yolk sac include calcification, very small yolk sac (<2mm) and irregular yolk sac. The calcified yolk sac almost indicates impending pregnancy loss and on the other hand irregular rim of yolk sac can be still associated with successful pregnancy outcome [15].

Figueras F *et al*. conducted a study to find the relation between yolk sac volume and risk of spontaneous abortion [16]. They found that yolk sac volume outside the 5th to 95th percentile were associated with significant occurrence of retrochorialhaematoma and subsequent pregnancy loss. In a prospective cohort study, abnormal yolk sac characteristics (yolk sac diameter outside the range of 2-5 mm, irregular shape, presence of degenerative changes, unequal number with embryo and presence of calcifications) were found in 22 first trimester ultrasound scans [17]. Abortion occurred in 14 (63.63 %) compared to 3.55 % (6/169) signifying the fact that normally functioning yolk sac is vital for the survival of the embryo. Our study too confirms the same findings.

Another study from Turkey prospectively evaluated sonographic characteristics of yolk sac in 305 viable singleton pregnancies with gestational age between 6 to 9 weeks and found abnormal yolk sacs in 66 patients **[15]**. An irregular yolk sac was observed in 78.8% (52/66), an enlarged yolk sac in 12.1% (8/66) and echogenic yolk sac in 9.1% (6/66). It was found that abortion occurred in 37.5% (3/8) of patients with enlarged yolk sacs and interestingly abortion rates were similar to normal population in patients with irregular yolk sacs and echogenic yolk sacs (3.8% and 1.5% respectively).

The development in the obstetric ultrasound 50 years ago mainly focussed on documentation of embryonic heart rate in the first trimester scan to confirm viability [18, 19]. Subsequently it was realized that slow embryonic heart rate was associated with increased rate of spontaneous abortions [7]. Now it is universally known that embryonic heart rate serves as one of the important predictors of imminent foetal demise [20, 21].Transvaginal ultrasonography has higher resolution and hence it can be used for visualization the embryonic heart beat in M mode. The embryonic heart rate can be visualized as early as 5 - 6 weeks of gestation and it is known that the mean heart rate progressively increases from 6 weeks (120 to 140 bpm) to 9 weeks (145 to 170 bpm) after which it slowly stabilizes to lesser heart rate for rest of the pregnancy [22]. It has been observed that embryonic heart rate less than 100 bpm (beats per minute) is associated with higher risk of miscarriage and the risk of embryonic demise almost touches 100 % when the rate is less than 80 bpm [9, 23].The rate of chromosomal abnormalities and structural abnormalities are significantly higher in surviving foetuses when they have slow heart beats [24]. In one study, genetic amniocentesis was

performed in 6 women with slow embryonic heart rates [25]. After karyotype analysis it was found that 2 foetuses had trisomy 21, which represented significantly higher incidence (33%) of aneuploidy. In our study too, we found higher rate of abortions with reduced heart rate, however we did not perform any karyotype analysis of the abortus or genetic amniocentesis.

The results of the present study indicates that first trimester fetuses with correctly sized and normally appearing yolk sacs and embryonic heart rate above 100 bpm are associated with very good chance of ongoing successful pregnancies.

A brief analysis of published studies regarding yolk sac and embryonic heart rate in comparison to our results are shown in Table 7 and 8.

Table 7: Studies comparing pregnancy outcome in relation to yolk sac abnormalities

Authors	Year	Study Design	Study population	No.	Outcome
Kurtz *et al*. [12]	1992	Prospective longitudinal	First trimester of pregnancy 6 to 10 weeks	212	Yolk sac abnormalities are present in low risk as well as high riskpregnancies, not necessarily mean occurrence of abortion.
Lindsay *et al*. [13]	1992	Prospective	First trimester less than 10 weeks	486	Yolk sac outside the range of two standard deviations one either side of the mean allows prediction of an abnormal pregnancy outcome.
Błaszczyk *et al*. [26]	2000	Prospective	5 to 12 weeks	136	Yolk sac greater than 7mm is associated with poor pregnancy outcome.
Makrydimas *et al*. [27]	2003	Prospective	6 to 10 weeks	668	The mean Yolk sac diameter is less in abortion group compared to ongoing pregnancies (4.7mm vs. 5mm), however not statistically significant. But Yolk sac: Gestational age ratio is significantly reduced in abortion group (3.2 vs. 4.6).
Figueras *et al*. [16]	2003	Prospective	6 to 10 weeks	125	Yolk sac volumes outside the 5th to 95th percentile are associated with significant occurrence of retrochorialhaematoma and subsequent pregnancy loss.
Roman *et al*. [28]	2004	Prospective	5 to 12 weeks	391	Yolk sac >9mm shows growth disorder of foetus or necrobiosis.
Cho *et al*. [29]	2006	Prospective	6 to 10 weeks	154	Absent yolk sac, deformed yolk sac and a large yolk sac (> 95%th percentile) are associated with anembryonic pregnancies.
Ivaniseviæ *et al*. [30]	2006	Case control study	60 women with type-1 diabetes, 60 healthy patients	120	Yolk sac diameter in type-1 DM women is significantly larger after 6 weeks of gestation when HbA1c levels are >6%.
Varelas *et al*. [31]	2008	Prospective	First trimester < 12 weeks	219	Absent or smaller yolk sacs are predictors of poor pregnancy outcome during the first 12 weeks.
Berdahl *et al*. [14]	2010	Retrospective Case Control study	Cases ≥ 5mm Yolk Sac And Controls 3-4mm Yolk Sac	175	Yolk Sac diameter (≥ 5mm) is associated with three fold increased risk of first trimester abortion.
Papaaioannou *et al*. [32]	2011	Prospective	729 miscarriages and 4698 normal pregnancies	5427	Yolk sac diameter above 95th centile is associated with 22.4% (163/729) chance of abortion vs. 5% (234/4968) in normal pregnancies.
Moradan *et al*. [17]	2012	Prospective cohort	First trimester at 5 to 6.5 weeks	193	Abnormal yolk sac characteristics (yolk sac <2mm &>5mm, irregular shape, degenerative changes and calcifications) are found commonly in miscarriages.
Tan *et al*. [15]	2014	Prospective observational	6 to 8 weeks	354	Yolk Sac diameter (≥ 5mm) is associated increased risk of miscarriage
Present study	2015	Prospective cross sectional	Low risk population at 6 to 9 weeks	280	99 % ongoing pregnancy rate when yolk sac is within 2mm - 5mm range and with normal appearance.

Table 8: Studies comparing pregnancy outcome in relation to embryonic heart rate

Authors	Year	Study Design	Study population	No.	Outcome
Laboda *et al*. [8]	1989	Prospective	Low risk population	65	100 % abortion rate with EHR < 85 bpm
Brown *et al*. [33]	1990	Prospective	CRL < 12 mm	398	Absence of cardiac activity with CRL > 5 mm indicates non viability
Tezuka *et al*. [34]	1991	Prospective	Low risk population	143	If EHR < 5th centile, 80 % abortion rates
May and Sturtevant [23]	1991	Prospective	4.5 to 7.3 weeks	50	54.5% (6/11) abortion rate with EHR < 85 bpm
Achiron *et al*. [20]	1991	Prospective cross sectional	Low risk population	603	If EHR < 5th centile, sensitivity 65%, False negative rate 35%
Benson and Doubilet [35]	1991	Prospective	EHR < 90 bpm, Gestation < 8 weeks	40	100 % abortion rate with EHR < 70 bpm, 80 % abortion rate with EHR < 90 bpm
Coulam *et al*. [36]	1996	Prospective	5 to 8 weeks	361	20 to 30 mm gestational sac must contain 2 to 5 mm embryo with EHR in 75 to 100 bpm range
Sefos *et al*. [37]	1998	Prospective	6 to 8 weeks	2164	100 % abortion rate with EHR < 85 bpm, EHR should increase linearly with gestation
Makrydimas *et al*. [27]	2003	Prospective	6 to 10 weeks	668	The mean EHR is less in abortion group compared to ongoing pregnancies (116 vs. 150 bpm)
Chittacharoen and Herabutya [6]	2004	Prospective cross sectional	Threatened abortion, live embryo	240	EHR < 120 bpm is associated with high risk of abortion
Doubilet and Benson [38]	2005	Prospective longitudinal	6 to 7 weeks	310	The rates of first-trimester demise = 60.6% for pregnancies with slow heart rates
Arleo and Troiano [39]	2011	Prospective longitudinal	5 to 6 weeks with EHR less than 100 bpm	36	High rates of spontaneous abortions (44.4%) and discordant growth (20%) in surviving foetuses
Papaaioannou *et al*. [40]	2011	Prospective	729 miscarriages and 4698 normal pregnancies	5427	EHR less than 5th centile is associated with 23.6% (174/729) chance of abortion vs. 5% (234/4968) in normal pregnancies.
Present study	2015	Prospective cross sectional	Low risk population at 6 to 9 weeks	280	83/3% (10/12) abortion rate with EHR < 100 bpm

CONCLUSION

Our data suggests that a normally appearing yolk sac measuring within the range of 2mm to 5mm reasonably ensures viability of pregnancy in the first trimester. However variation outside this range does not necessarily mean adverse outcome as in more than 50% of cases, pregnancy still can continue. On the contrary the same is not true for embryonic bradycardia as it can suggest pregnancy failure in more than $4/5^{th}$ of cases. Hence it is important to monitor embryonic heart rate in first trimester pregnancy complications and presence of normal cardiac heart rate may guide treating obstetrician in patient counseling and planning further management strategies. Though in our study the number of patients with spontaneous abortions appears small, sample size was significant enough to draw important conclusions. Many a times, by the time patient presents with vaginal bleeding, they would have had either failing embryonic heart or missed abortion and this can limit the sample size as our study population included low risk antenatal women without any symptoms. Ideally all patients who have missed their periods should have regular ultrasound examination right from 5^{th} week of gestation and examination should be repeated at regular intervals if any abnormalities of yolk sac and embryonic heart rate are detected. If universal early first trimester sonographic screening is not feasible, at least those women with bad obstetric history such as recurrent abortions, previous history of congenital or chromosomal abnormalities and those with endocrine disorders should be offered routine ultrasound screening right from the first trimester of

pregnancy to forecast any possible untoward adverse foetal outcomes.

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