

ULTRASOUND MEASUREMENT OF FETAL ADRENAL GLAND VOLUME IN PREDICTING SPONTANEOUS ONSET OF LABOUR

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ABSTRACT:

BACKGROUND: The Placental clock plays a pivotal role in interrupting the uterine quiescence and leads to onset of labor^(1,2). Biochemical activation causes increased dehydro-epiandrosterone sulfate production in central zone of fetal adrenal gland. Hence fetal adrenal zone and adrenal gland increase in size This imparts a clue that fetal adrenal gland measurement in pregnancy can be used as a non invasive marker for onset of labor and hence found to be useful in predicting preterm labor^(3,4). ⁽⁴⁾Dimensions of fetal adrenal gland and fetal adrenal zone increase with increasing gestation age as part of physiological development. However despite this increase, proportion of gland occupied by fetal zone remains constant throughout gestation in all dimensions. This facilitates the distinction between an abnormal hypertrophy of fetal zone and a physiologic increase in its size that is in linear relationship with remainder of gland⁽⁴⁾. More number of women are interested in prediction of spontaneous onset of labour even at term. In recent scenario cervical assessment is used for same. In our study we hypothesise that fetal adrenal gland volume and adrenal zone parameters can predict preterm and term labor with same efficacy. **METHODOLOGY:** A prospective observational cohort study with low risk pregnant women is conducted. The subjects underwent a detailed clinical examination. Apart from routine investigations, they were assessed for corrected fetal adrenal gland volume, fetal adrenal zone parameters including fetal adrenal zone width ratio and zone depth ratio and cervical length at 28 to 34 weeks period of gestation. Corrected fetal adrenal gland volume and adrenal zone parameters was assessed again at 37 to 39 weeks period of gestation. Data is recorded in specially designed proforma and then transferred to master sheet. **SUMMARY:** In a prospective study conducted in KGH, with a 100 sample size, majority of patients were primigravida, less than 25yrs. A significant correlation was found between increased fetal adrenal gland and fetal adrenal zone enlargement in those who delivered preterm. Enlarged fetal adrenal gland volume of 331 mm³/kg and enlarged fetal width ratio are an excellent predictor of preterm delivery in this study when compared to depth ratio and cervical length. Also this study found that fetal adrenal gland volume enlargement can predict the occurrence of spontaneous labour. On followup scan at term gestation of 37 to 39 weeks, spontaneous labour group had more adrenal gland volume when compared to those who had to be induced. **CONCLUSION:** The concept that the fetus contributes to the start of parturition is the basis for the usage of fetal adrenal gland volume and fetal adrenal zone enlargement to forecast the likely occurrence of preterm birth and also to predict the spontaneous onset of labour at term. The same has been showed in this study where women who delivered preterm and in those who had spontaneous term labour had increased fetal adrenal gland volume and fetal adrenal zone enlargement. It would be helpful in incorporating fetal adrenal gland volume in our regular follow-ups. In those with threatened preterm and enlarged fetal adrenal gland we can avoid unnecessary intervention like cervical encirclage or tocolysis and such women can be given antenatal steroids and referred to a tertiary center with NICU and SNCU care. In modern obstetrics, the establishment of a day-by-day predictable cutoffs would provide fetal adrenal glands a new role in the prediction of labour (term or preterm). In all women prior to induction of labour, we can consider AGV as a part of routine ultrasonography to predict successful induction of labour. This sort of predictability not only will offer the obstetrician time to efficiently employ resources but also will provide the parturient and her family a clarity of plan.

Keywords: FETAL ADRENAL GLAND VOLUME

INTRODUCTION:

Nowadays, we see an increase in the number of women who are interested to know the possibility of predicting spontaneous onset of labour. In current situation, a clinical cervical examination or ultrasonographic cervical length measurement is done to predict preterm labour along with numerous biochemical markers which are available to predict preterm onset of labour like fetal fibronectin, cervical interleukin-6. But there is an increasing demand for a marker to predict spontaneous onset of both term and preterm labour. In recent studies, there is an increasing evidence that human fetus provides signal to initiate parturition.⁽¹⁾This ability of fetus to send endocrine signaling to initiate parturition has been studied. Trials are being done to find a highly sensitive, specific, non-invasive and cost-effective marker to predict spontaneous onset of labour at term and preterm. This study hypothesizes about such a marker which is Fetal adrenal gland volume that can be used to predict spontaneous onset of labour based on theory of "Placental clock".

In **Smith et.al study**, they suggested that timing of parturition is associated with increased expression of corticotrophin-releasing hormone from placenta. This placental corticotrophin releasing hormone further stimulates maternal and fetal pituitary glands to produce adrenocorticotropin hormone, which results in fetal adrenal gland enlargement due to increased stimulation to release cortisol.⁽²⁾ This follows a series of biochemical reactions that leads to initiation of parturition with release of estrogen, prostaglandin and oxytocin. Thus placental clock plays a crucial role in removing the mechanisms maintaining uterine quiescence and initiating a cascade of events resulting in labour. This knowledge can be used to assess fetal adrenal gland volume as a non-invasive marker to predict spontaneous onset of labour. Our study assesses the efficiency of fetal adrenal gland volume in predicting preterm labour which is still a major health problem in the world, accounting to second most common cause of neonatal mortality of 28%. Although 30-35% of preterm births are due to medical and obstetric reasons, 30-35% are due to preterm premature rupture of membranes, remaining 40-45% are due to spontaneous preterm labour⁽³⁾. Structure of fetal adrenal gland is different from its adult counterpart. In utero, fetal zone is most prominent and distinctive portion of adrenal gland. Consequently, fetal zone occupies majority of adrenal gland size. Fetal adrenal gland undergoes significant growth because of increase in fetal zone size. Hence, in our study we hypothesise that fetal adrenal gland volume as well as adrenal zone parameters can predict preterm and term labour with same efficacy. By analysing the degree of adrenal gland

enlargement and calculating cervical length, this physiological cascade can be indirectly used to predict PTB. These quantitative indicators have previously been explored in isolation, but no comparison has been made. The goal of this study was to see how accurate fetal adrenal gland enlargement and cervical length were at predicting preterm birth.

AIMS & OBJECTIVES:

1. Comparison of corrected fetal adrenal gland volume and adrenal zone parameters in women who had preterm delivery and those who progressed till term in study population.
2. Comparison of efficacy of corrected fetal adrenal gland volume, adrenal zone parameters and cervical length for prediction of preterm delivery in study population.
3. To study adrenal volume and adrenal zone parameters among two groups of women who reached till term (spontaneous versus those who had to be induced).

REVIEW OF LITERATURE:

Term labour might be seen of as a physiological release from the myometrium's inhibitory effects during pregnancy, rather than an active process driven by uterine stimulants. Activation of labour owing to infection, inflammation, or other pathologic conditions, which short circuit or overwhelm the normal parturition cascade at term, appears to be the cause of many occurrences of premature labour.

CASCADE OF PARTURITION:

At term, there is likely to be a "parturition cascade" that removes the mechanisms that maintain the uterus quiet and recruits factors that increase uterine activity^[4]. Such a cascade definitely includes numerous redundant loops to guarantee a fail-safe method for ensuring pregnancy success (and thus preserving the species). Many of such factors involved are connected to each other in a sequential manner and demonstrate a positive feedback mechanism resulting in a cascade of events. Because of the successive influx of signals that help to boost the labour process, it's probable that no one signalling pathway is accountable for the onset of labour. As a result, such processes should be described as "boosting" rather than "initiating" the labour process.

ROLE OF FETUS IN INITIATION OF LABOUR:

Most viviparous species have substantial evidence that the fetus regulates the beginning of labour^[5]. The fetus was supposed to be positioned head down at term so that it could kick its legs up against the fundus and drive itself through the birth canal during the Hippocratic period. Initial research concentrated on endocrine events,

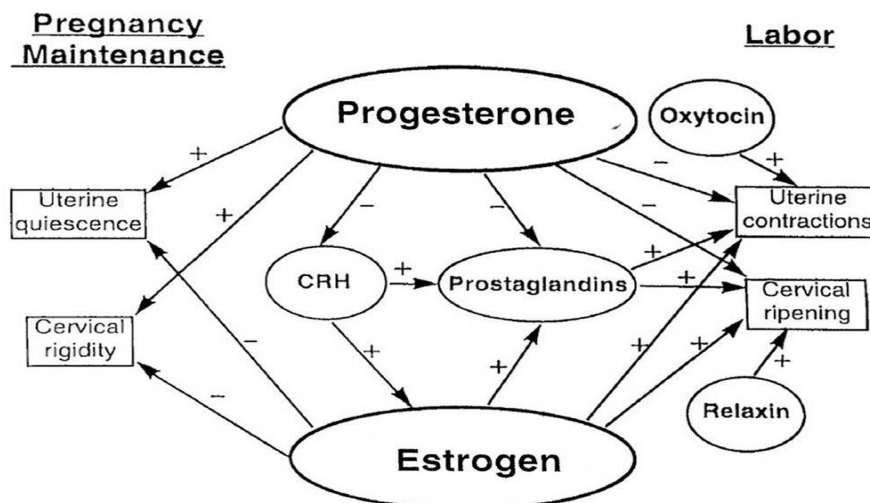
such as changes in the profile of hormone levels in the maternal and fetal circulations, but this leaves little information regarding the endocrine milieu at the maternal-fetal interface, where parturition occurs. Following research have focused on the fetus and mother's dynamic biochemical dialogue (paracrine/autocrine processes) in an attempt to understand the molecular mechanisms that control such interactions at the uterine level. The genetic control of parturition-related molecular processes is also being explored. In domestic ruminants, the concept that the fetus controls labour time has been eloquently proved [6]. Parturition in sheep, for example, is triggered by a sudden increase in fetal adrenal cortisol production, which is linked to enhanced fetal corticotropin concentrations and responsiveness [7]. Cortisol stimulates estrogen secretion while suppressing progesterone production by acting on placental enzymes involved in the manufacture of estrogens from progesterone. The increased estrogen- to-progesterone ratio boosts placental production of prostaglandin F2 alpha, which improves myometrial oxytocin response and induces contractions. The glucocorticoid-inducible 17-alpha-hydroxylase/ 17,20- lyase enzyme, which is required for this pathway, is missing from the human placenta[8,9]. As a result, in humans, activation of the hypothalamic-pituitary- adrenal axis is expected. Parturition is a sequential, integrated set of changes within the myometrium, decidua, and cervix that occurs gradually. In humans, labour is triggered by a complicated dynamic biochemical dialogue between both the fetoplacental unit and the mother (paracrine and autocrine events). The four physiologic phases of uterine activity during pregnancy are inhibition, activation, stimulation, and involution[10].

Phase 0: Inhibition - The myometrium is in a state of functional quiescence during the majority of pregnancy due to the activity of many potential inhibitors, not all of which are limited to: Progesterone, Prostacyclin (prostaglandin I2), Relaxin, Parathyroid hormone-related peptide, Nitric oxide, Calcitonin gene-related peptid, Adrenomedullin, Vasoactive intestinal peptide

Phase 1: Myometrial activation (priming). Near to term, progesterone action in the uterus is functionally revoked, and an increase in uterotropin levels, such as estrogen, causes myometrial activation by increasing expression of a series of contraction-associated proteins (CAPs; including prostaglandin and oxytocin receptors in the myometrium), activating specific ion channels, and increasing connexin-43 (a key component of gap junctions). This allows more gap junctions to form between adjacent myometrial cells, culminating in electrical synchrony within the myometrium and enhanced contraction coordination.

Phase 2: Following myometrial activation, endogenous and exogenous uterotonic agonists, such as the stimulatory prostaglandins E2 and F2 alpha and oxytocin, stimulate the primed uterus to contract, resulting in delivery.

Phase 3: Involution - Following birth, the uterus involutes. Oxytocin is the main mediator in this process. Control of pregnancy and parturition in women is through endocrinology. The balance of estrogen and progesterone effects is crucial for the continuation of pregnancy and the commencement of labour. As illustrated in the diagram, several significant hormonal elements influence this equilibrium.



Excitability in Uterine Smooth Muscles and Coordination of Myometrial Contractility:

Role of Hormones:

Action potentials, which might be simple spike or complex spike, signal myometrium contractility. L-type calcium channels, transient sodium channels, and quickly activating and inactivating calcium channels are all thought to be responsible for simple spikes in human myometrium.^[11] Simple spikes are followed by a depolarization plateau that lasts for a long time in complex APs. This type of AP is particularly noticeable in the inner layer and top segment (fundus) of the uterus, and it occurs throughout the third trimester and labour.^[12] The duration of the plateau determines the length of the contraction^[13,14]. The action potential calcium wave theory is thought to be responsible for rapid AP propagation throughout the uterus^[15].

The following are important aspects of this hypothesis:

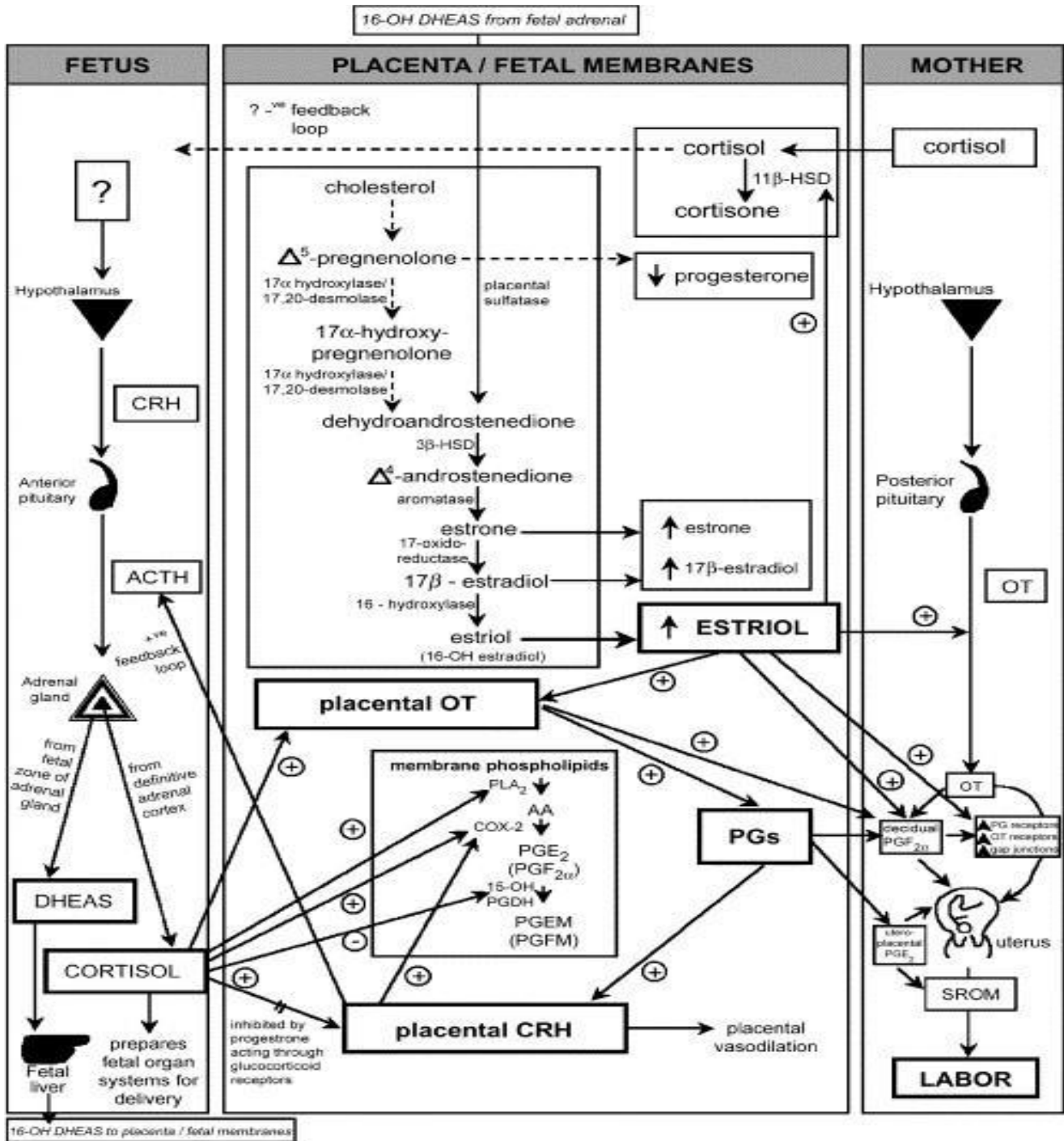
- Intercellular calcium waves are initiated by APs propagating through the uterus. This stage synchronises the start of the contraction across the thickness of the uterus's wall and across all of its regions.
- An intercellular calcium wave propagates across each bundle after being initiated by an AP, and myocytes contract individually as the wave passes. Calcium waves do not spread across bundle borders. Intercellular calcium wave propagation requires either functioning gap junctions or paracrine signalling pathways.
- The direct recruitment of myocytes for contraction does not need electrical activity; but, gap junction function is necessary for the action potential to propagate across each bundle in the uterus.
- Each myocyte stays contracted for as long as the $[Ca^{2+}]_i$ is raised, which is governed by the calcium metabolism of each cell.

In response to depolarization, prostaglandin F₂ and oxytocin stimulate the opening of L-type calcium channels. Estrogen has a role in the transformation of AP

into complex forms. As the pregnancy progresses, oxytocin increases the plateau component of complex APs, resulting in a steady increase in the length of contractions.

MYOMETRIAL ACTIVATION:

A fetal endocrine cascade involving the fetal H-P-A axis triggers the commencement of labour in most species, resulting in an increase in estrogen and a drop in progesterone in maternal plasma^[16]. The myometrium is both 'activated' (contraction-associated protein (CAP) expression) and 'stimulated' (production of uterotonic agonists such as oxytocin and stimulatory prostaglandins) as a result of this endocrine cascade [Figure 3]. Increased expression of the gap junction protein connexin-43 (Cx-43), as well as the oxytocin receptor (OTR) and prostaglandin F receptor, is linked to activation. These CAPs are favourably controlled by estrogen and negatively regulated by progesterone, and CAP expression is enhanced in preterm labour but not when progesterone blocks labour^[17]. Other CAPs, including the sodium and calcium channels, see a rise in expression closer to term. Other putative CAPs expressed in the uterus include enzymes that regulate uterotonic levels (oxytocin endopeptidase and cyclooxygenase), proteins that interact with actin/myosin (e.g., MLCK, calmodulin), and other uterotonic receptors (e.g., endothelin, thromboxane A₂, -adrenergic and potassium channels), but there is Cx-43 and OTR gene transcription which is increased by estrogen. In the myometrium, estrogen raises the amounts of mRNA encoding the AP-1, protein, c-fos, which occurs before the enhanced expression of Cx-43. In the rat, increased expression of c-fos as well as Cx-43, is related with the start of term and preterm labour, and expression of these genes is suppressed when labour is prevented by progesterone. Stretch-induced gene expression in the myometrium can be blocked by progesterone, a vital pregnancy-maintaining hormone, and myometrial growth can be maintained post-term^[18].



At term, a parturition cascade leads to labour induction. Paracrine and autocrine elements acting in harmony enhance uterine contraction during induction of labour at term. COX-2: Cyclooxygenase 2, OT: Oxytocin,

MATERIAL AND METHODS:

STUDY SITE: The present study was carried out in the Department of OBSTETRICS AND GYNAECOLOGY of King George Hospital, Visakhapatnam

STUDY POPULATION: All pregnant women attending Department of obstetrics and Gynecology, KGH, Visakhapatnam.

STUDY DESIGN: This is a Prospective observational

study.

SAMPLE SIZE: 100 women.

STUDY DURATION : November 2019 to October 2021

INCLUSION CRITERIA:

- Pregnant women aged >18yrs.
- All pregnant women 28 to 34 weeks of gestation and those who progressed to 37 to 39 weeks period of gestation.
- Pregnant women who presented with features of preterm labour.
- All singleton pregnancies.
- Cephalic presentation.
- Pregnancies with gestation age calculated according to Last Menstrual Period and correlated with early scans.
- Women who gave consent for study.
- Who are willing for follow up and planning to come to delivery at King George Hospital, Visakhapatnam.

EXCLUSION CRITERIA:

- Multiple gestation
- Premature rupture of membranes.
- GDM
- Gestational hypertension or pre eclampsia
- IUGR
- Placental previa
- Infections
- Abnormal presentation
- Congenital Anomalies
- Women who did not give consent for study.

METHODOLOGY:

The Institute ethics committee approval will be obtained. Written and informed consent will be obtained. A prospective observational cohort study with low risk pregnant women (excluding risk of preterm labour only) will be conducted. The subjects will undergo a detailed clinical examination. Apart from routine investigations, they will be assessed for corrected fetal adrenal gland volume, fetal adrenal zone parameters including fetal adrenal zone width ratio($w/W = \text{Fetal adrenal zone width} / \text{Total adrenal gland width}$), zone depth ratio($d/D = \text{Fetal adrenal zone depth} / \text{Total adrenal gland depth}$) and cervical length in first scan at 28 to 34 weeks period of gestation. Corrected fetal adrenal gland volume and adrenal zone parameters will be assessed in second scan at 37 to 39 weeks period of gestation. Data is recorded in specially designed proforma and then transferred to master sheet.

ULTRASOUND PROTOCOL:

Antenatal women will be subjected to 2D Ultrasound

after clinical evaluation according to following protocol.

A. ADRENAL GLAND:

- All examinations will be performed using SAMSUNG RS80A MODEL- S23CMU1HS in Department of Radiodiagnosis, Andhra medical college, Visakhapatnam.
- 2D ultrasound study will be performed to determine fetal weight using biparietal diameter, head circumference, abdominal circumference, and femur length.
- To determine the Adrenal gland volume, settings like dynamic range, gain, frame rate will be standardized on the device to obtain the best contrast between the Adrenal Gland and adjacent structures.
- The fetal abdomen was positioned so that the fetal spinal column was located between 2 and 4 o'clock or between 8 and 10 o'clock position to facilitate identification of the Adrenal glands.
- Fetal adrenal gland is visualized as a hypo echoic, inverted 'V-shaped structure/cap-like structure above kidney.
- The fetal adrenal zone is visualized as hyper echoic center in the fetal adrenal gland. Fetal Adrenal gland and fetal zone will be identified in transverse, sagittal planes.
- These planes were used to measure the length, width, and depth of the adrenal glands and their fetal zones.
- Measurements were obtained from both adrenal glands in those planes and the median of both adrenal glands measurements were recorded.
- All measurements were made in millimeters and the three repeat measurements were averaged and stored for the analysis

INTERPRETATION OF DATA:

- The fetal adrenal gland volume was calculated using the ellipsoid formula ($0.523 \times \text{length} \times \text{width} \times \text{depth}$).
- Corrected fetal adrenal gland volume (cFAGV) was obtained from dividing fetal adrenal gland volume by the estimated fetal weight, so as to make it a gestational-age independent factor.
- Fetal adrenal zone length and width including Fetal adrenal zone depth ratio and width ratio are measured.
- The subjects were then followed until delivery.
- Mode of delivery and onset of delivery will be recorded.
- The gestational age at delivery, birth weight were recorded and results will be interpreted.

B. CERVICAL LENGTH:

- To measure the cervical length, standard criteria were followed. A transvaginal probe (5-9Hz) was used and closed portion of cervix from internal os to external os was measured.
- Cervical length and adrenal gland parameters are compared in 28 to 34 weeks period of gestation to predict

preterm labour.

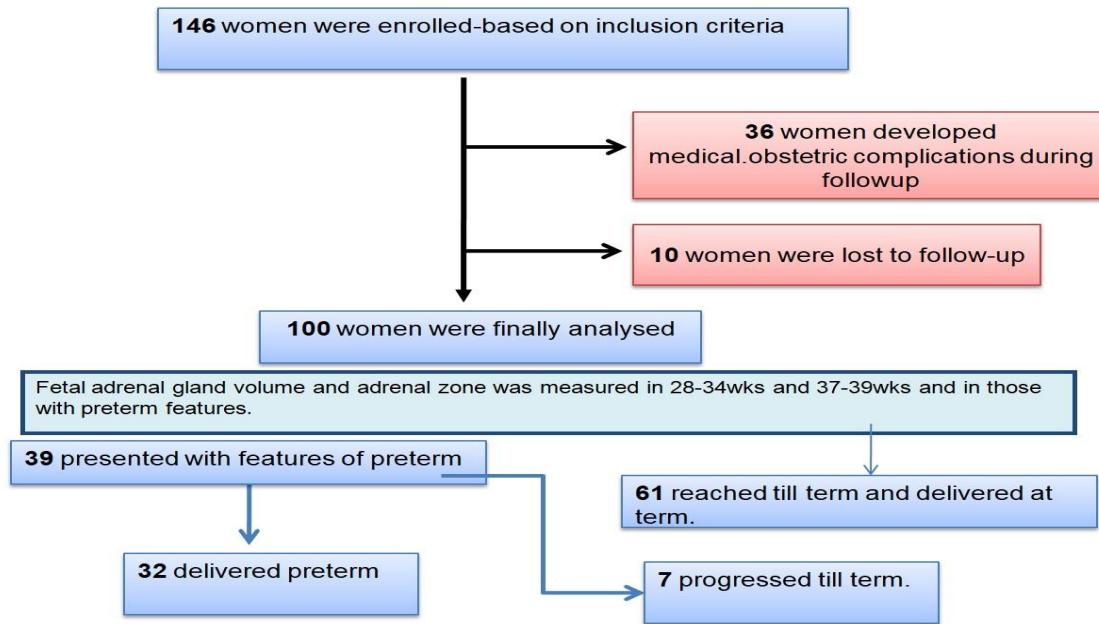
STATISTICAL ANALYSIS:

- Date obtained will be entered using M.S Excel and it will be statistically Analyzed using statistical package

for social sciences (SPSS Version 16) for M.S windows.

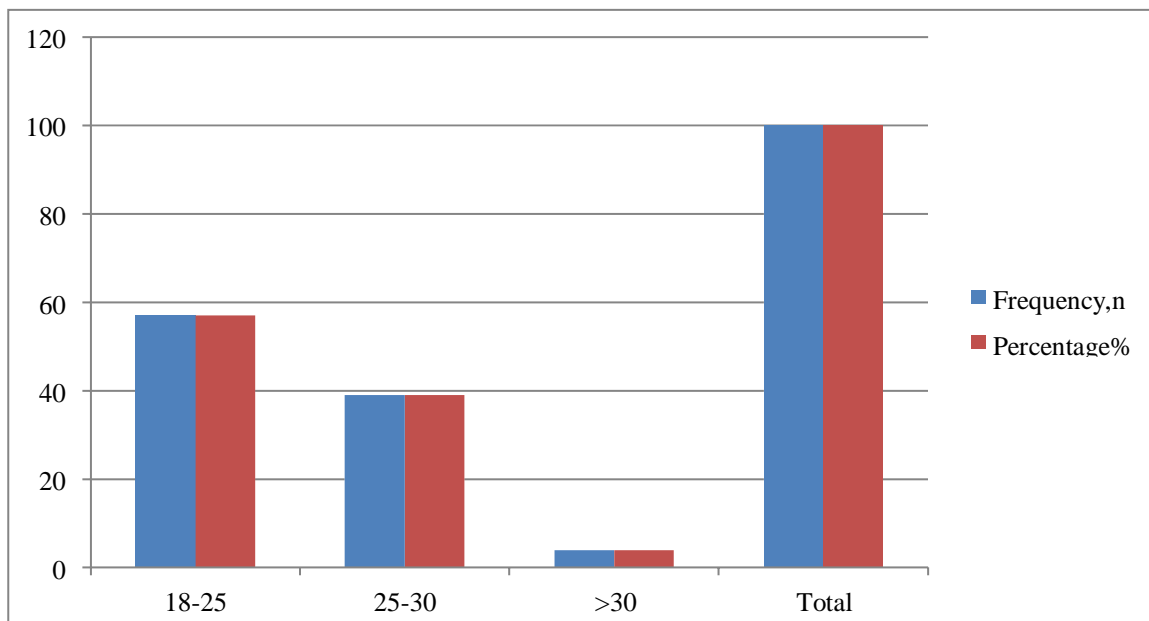
- Descriptive statistical analysis will be carried out. All results will also be presented in tabular form and also in graphically using bar diagram or pie diagram as appropriate.

RESULTS & OBSERVATIONS:

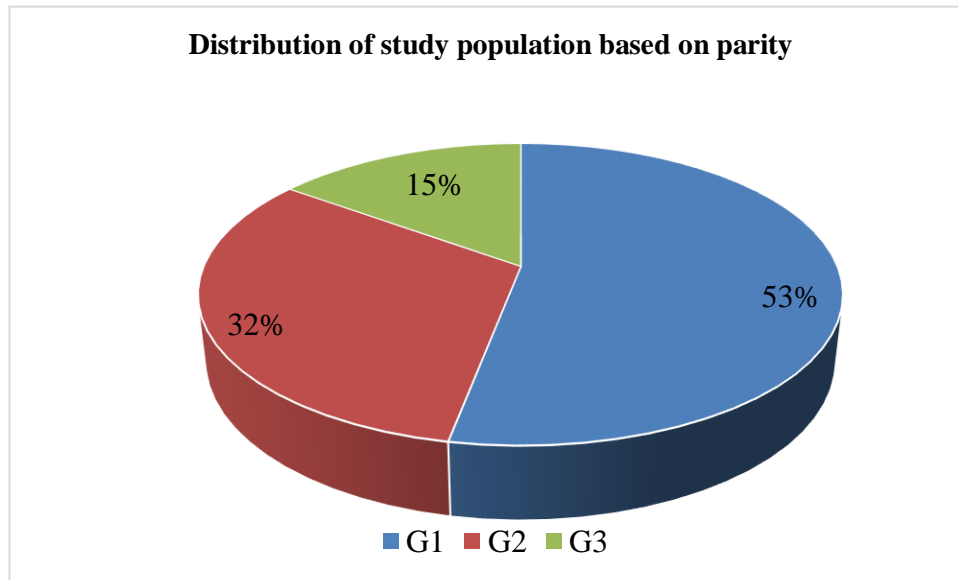


Graph 1:Distribution of study population according to age Categories:

Majority of the patients belong to the age group of 18-25 years (57%) followed by 25-30 years (39%),>30 years(4%).



Majority of study population are primi gravida (53%).



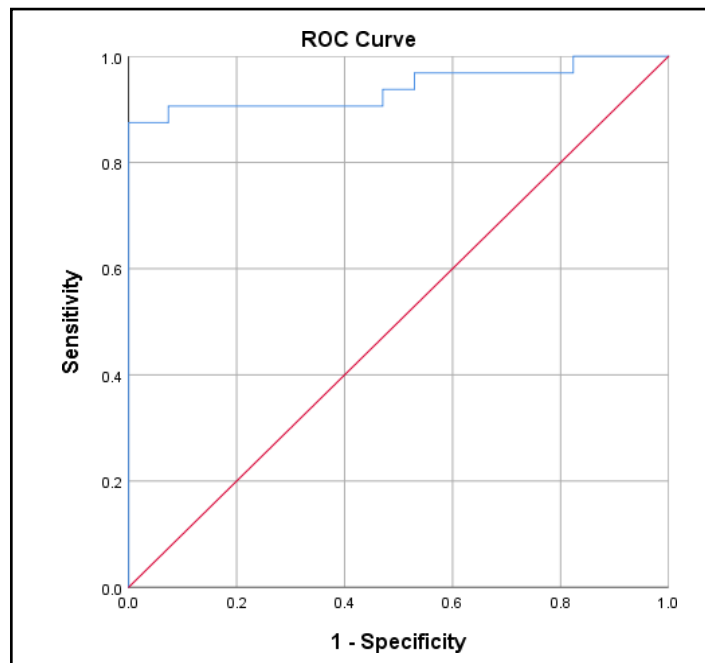
Graph 2: Distribution of study population based on parity.

Table 1: Distribution of study population-based on Gestational Age at Delivery:

GA at Delivery	Frequency,n	Percentage%
Preterm	32	32
Term- spontaneous	55	55
Term- Induced	13	13
Total	100	100

ROC Curve for Prediction of **PRETERM DELIVERY** using Adrenal Gland volume, width ratio and depth ratio at first scan:

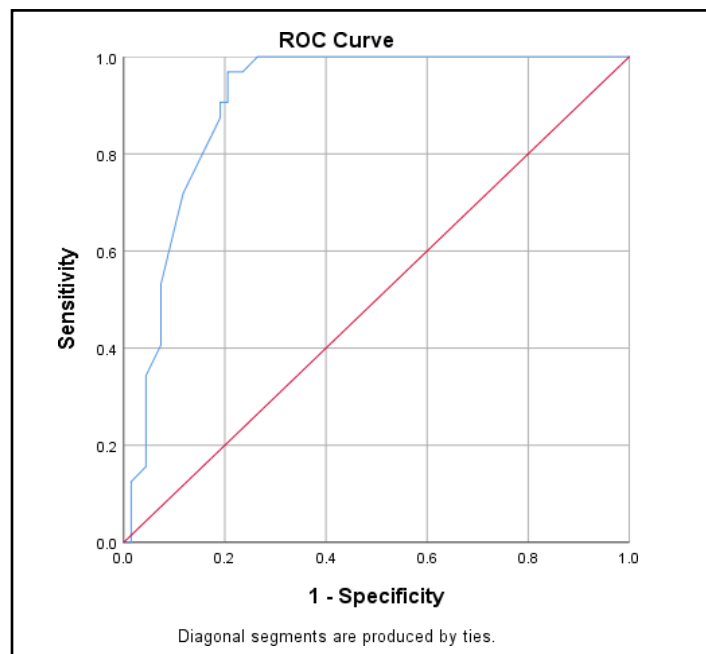
1. Adrenal gland volume (CFAGV (mm³/kg)) vs Preterm delivery:



Area Under curve for **Adrenal Gland Volume** was found to be 0.94 which indicates that it is an **Excellent predictor**

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.941	0.033	0.000	0.87	0.98
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

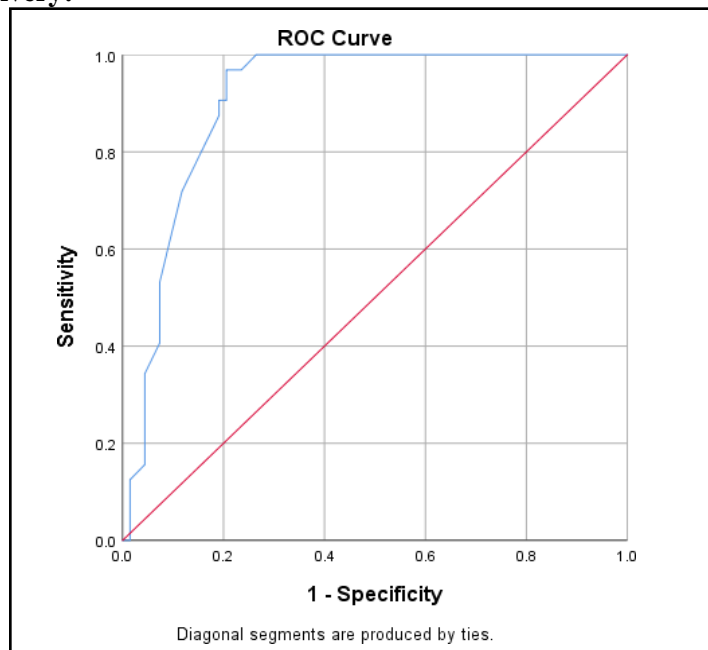
2.Width ratio vs Preterm delivery:



Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.907	0.029	0.000	0.850	0.965
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Area Under curve for **width ratio** was found to be 0.90 which indicates that it is an **Excellent predictor** for preterm delivery .

3. Depth ratio vs Preterm delivery:



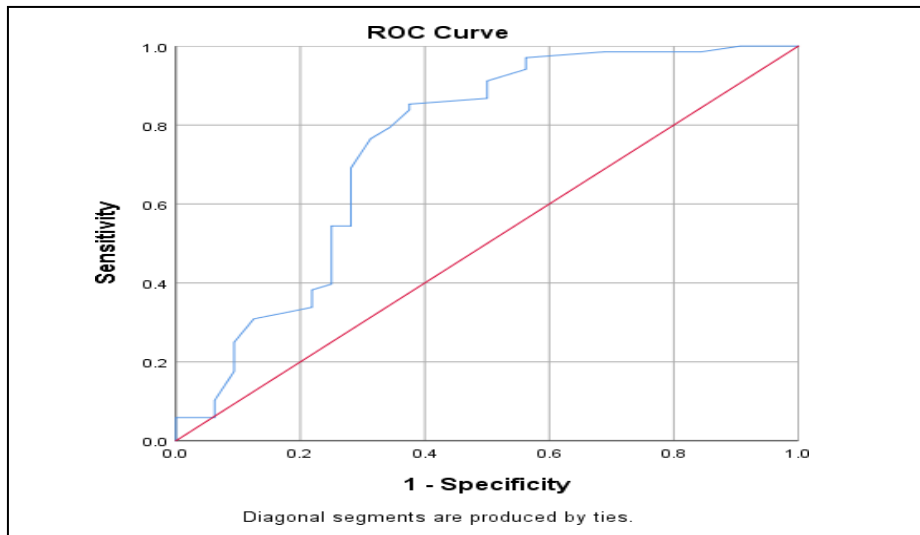
Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.787	0.048	0.000	0.693	0.881
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Area Under curve for **depth ratio** was found to be 0.78 which indicates that it is a **fair predictor** for preterm delivery.

Table 2: Comparison of efficacy of fetal adrenal gland with cervical length.

Statistical characteristics	cFAGV (331.04)	Width ratio (w/W)	Depth ratio (d/D)	Cervical length (</3.1cm)
Sensitivity	87.50	96.8	96.88	85.2
Specificity	98.53	79.4	75.00	62.5

4. ROC curve for Prediction of preterm delivery using Cervical length:

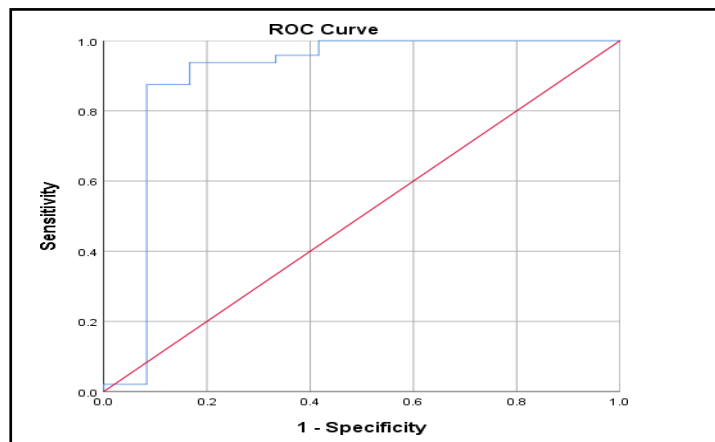


Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.746	0.060	0.000	0.630	0.863

Area Under curve for **cervical length** was found to be 0.74 which indicates that it is a **fair predictor** for preterm delivery.

ROC Curve for Prediction of **TERM DELIVERY** using Adrenal Gland volume, width ratio and depth ratio:

1. Adrenal gland volume (CFAGV (mm³/kg)) vs Term Spontaneous delivery:

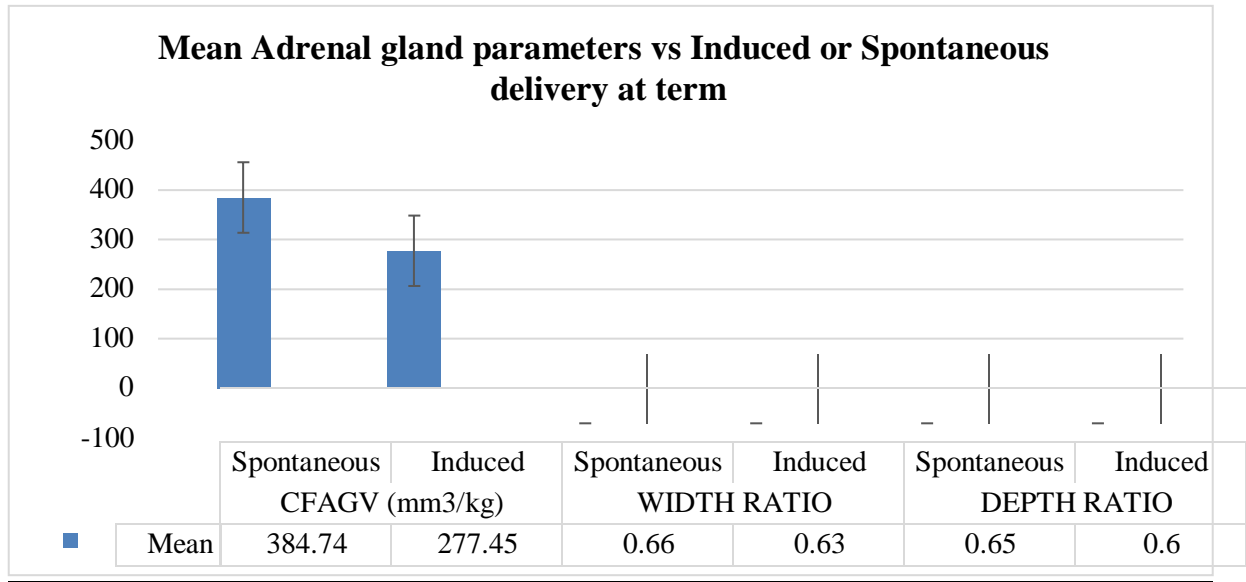


Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.894	0.078	0.000	0.742	1.000

a. Under the nonparametric assumption

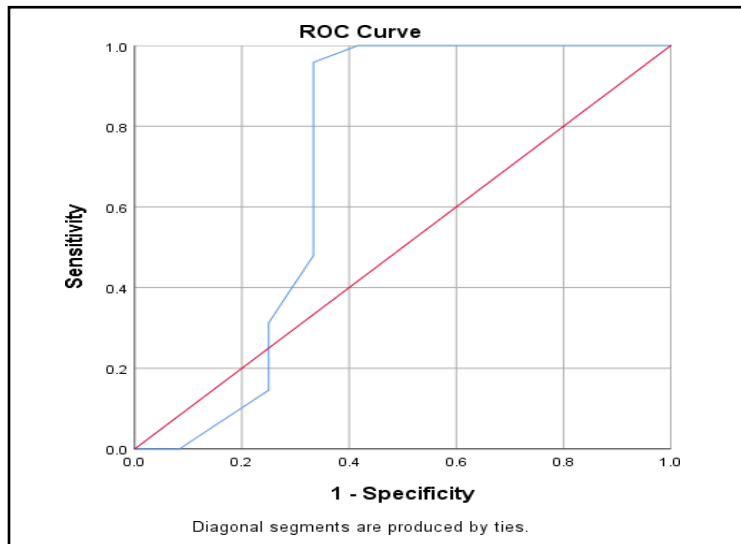
b. Null hypothesis: true area = 0.5

Area Under curve for **Adrenal Gland Volume** was found to be 0.89 which indicates that **good predictor for spontaneous labour** .



ROC Curve for Prediction of term Spontaneous delivery using Adrenal Gland volume, width ratio and depth ratio at Second scan:

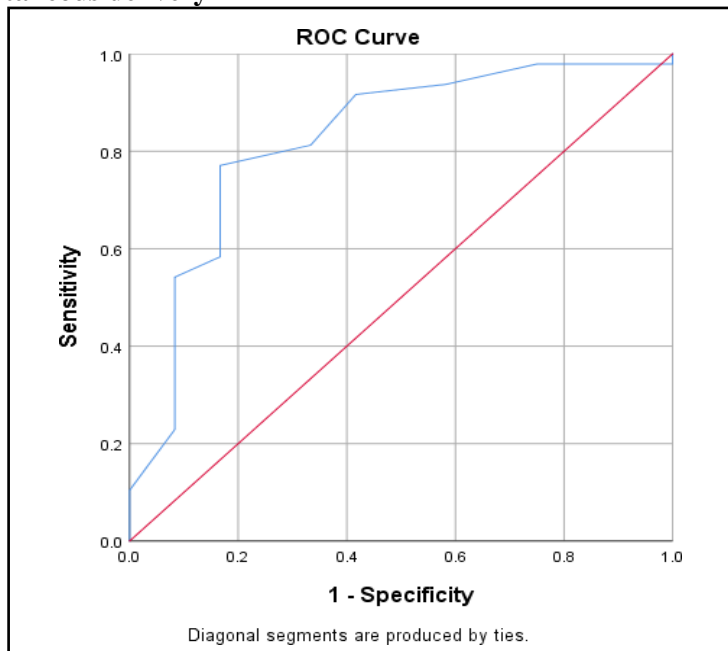
2.Depth ratio vs Term Spontaneous delivery:



Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.710	0.121	0.025	0.473	0.947
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Area Under curve for **depth ratio** was found to be 0.71 which indicates that **fair predictor for spontaneous labour**.

3.Width ratio vs Term Spontaneous delivery



Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.824	0.073	0.001	0.681	0.967
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Area Under curve for **width ratio** was found to be 0.82 which indicates that **good predictor for spontaneous labour**

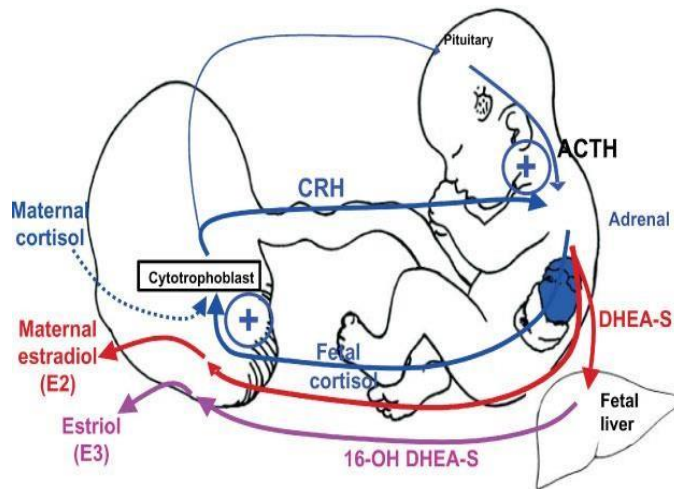
DISCUSSION:

The concept of a 'placental clock' underpins the use of fetal adrenal gland characteristics to anticipate the beginning of labour. This study provides a compelling evidence that the discovery of an enlarged fetal adrenal gland by 3D ultrasonography can be a highly accurate and noninvasive marker of preterm birth, based on previous findings that the onset of birth is initiated through the fetal genome and expressed via fetal endocrine pathways. Our motivation of this study is that the fetus itself may determine the time of birth by stimulation of its own hypothalamic-pituitary- adrenal axis, according to the many processes implicated.

penetrates both maternal and fetal circulation, stimulating the pituitary glands to create adrenocorticotropin hormone, which causes the adrenal cortex to release cortisol in both the maternal and fetal bodies. Unlike the negative feedback of cortisol on hypothalamic CRH, cortisol induces placental synthesis of corticotropin-releasing hormone in a positive feed forward feedback system.⁽⁷⁹⁾ Labour begins after a series of biological processes, including the production of prostaglandins and oxytocin.

PLACENTAL CLOCK:



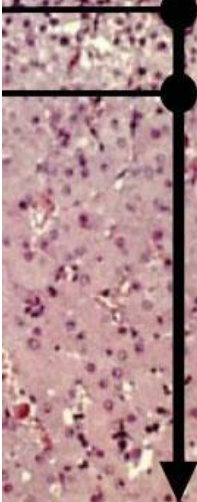
According to Smith et al. ^(2, 78), the timing of parturition is linked to gene expression and the placenta's exponential synthesis of corticotropin-releasing hormone. Placental corticotropin-releasing hormone

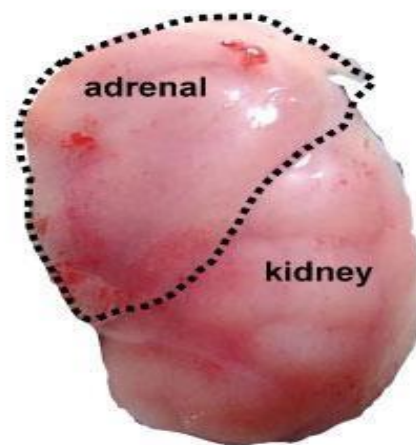
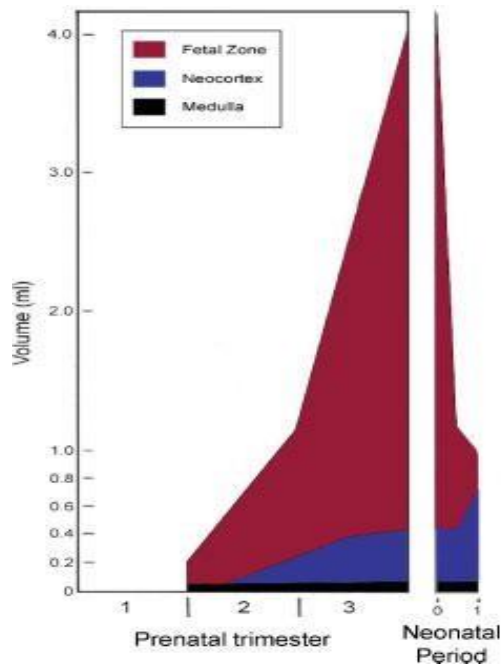


The endocrine cascade between the placenta, the foetus, and the adrenal glands. Rising levels of placental-derived CRH boost fetal adrenal DHEA-S and cortisol synthesis in late pregnancy. Increased availability to maternally generated cortisol, as well as fetal adrenal cortisol, increases placental CRH synthesis, resulting in a feed-forward loop that boosts adrenal steroid hormone production.

Fetal adrenal zone comprises of 3 zones namely:

1. OUTER DEFINITIVE ZONE=proliferative zone
2. MIDDLE TRANSITION ZONE = unique to humans ,in second trimester it increases cortisol production.
3. INNER FETAL ZONE=DHEAS production

	Fetal adrenal zones	Steroid products	Fetal functions
	Definitive zone	Aldosterone	Initiated late in gestation to control neonatal mineral balance
	Transitional zone	Cortisol	Increases in late gestation to promote organ maturation and assist in timing of parturition
	Fetal zone	DHEA-S and Preg-S	DHEA-S acts as precursor for placental estrogen production. Late in gestation DHEA synthesis increases and may act to help the timing of parturition



Fetal Adrenal/Kidney at 18 weeks gestation

The *human embryonic adrenal gland* differs greatly from its adult counterpart in anatomy. The fetal zone takes up the majority of the size of the adrenal gland. The fetal adrenal gland grows rapidly due to a rise in the fetal zone size, and by 18 weeks of pregnancy, the gland is nearly as big as the kidney. The fetal zone continues to expand, especially in the last six weeks of pregnancy.

The importance of the prenatal hypothalamic-pituitary-adrenal (HPA) axis was first demonstrated in a sheep model, where fetal hypophysectomy results in fetal adrenal atrophy and prevents premature delivery (PTB). Later, it was shown that continual maintenance adrenocorticotropic hormone (ACTH) infusions to raise blood cortisol levels in ovine fetuses caused premature parturition. These results suggest that ACTH is important for adrenal cortical development and maturation late in pregnancy, as seen by rising baseline cortisol levels before to delivery. A disproportionate fetal zone expansion has been demonstrated to indicate an acute premature activation of the parturition mechanism and to predict spontaneous PTB. When comparing neonates born in the situation of idiopathic PTB to those delivered due to fetal/maternal haemorrhage, an **autopsy investigation** indicated a substantially higher risk of PTB in fetuses with larger volume of the total fetal adrenal gland.⁽⁸⁰⁾

In our study, 100 women had their first ultrasound measurement of cFAGV between 28 to 34wks.

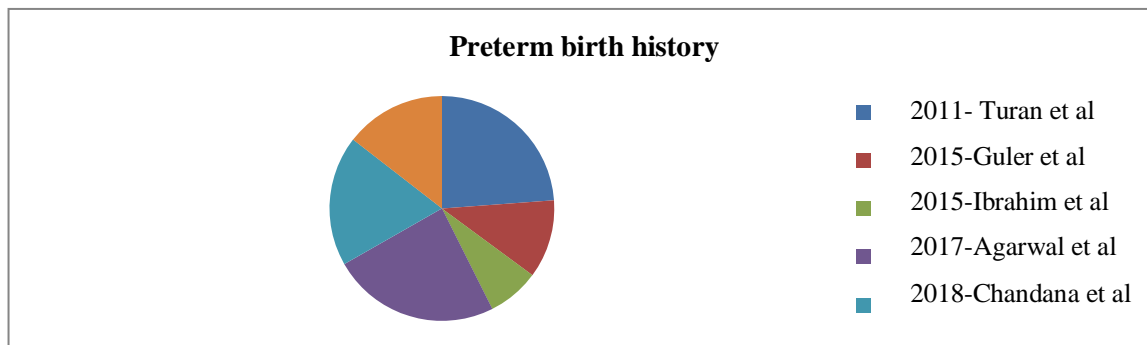
Among those 39 women, 32 delivered preterm and we excluded PPROM. A history of past preterm birth (PTB) is widely documented to be a risk factor for a preterm birth. (81) .

Studies have shown that if a patient has a history of past PTB, the likelihood of PTB before 37 weeks increases by up to 64 percent. (82) However, Prior PTB history did not predict the patients who would deliver within 7 days in the high-risk trial cohort of **Turan et al study** conducted in 2011. In **Guler et al study** conducted in 2015, only 4 out of 29 women (14%) who delivered preterm had history of preterm birth. In a study conducted by **Ibrahim et al**, similar result was seen. Only 7 out of 75 women who delivered preterm (9.3%) had prior history of preterm delivery. Whereas **Agarwal et al study** in 2017, found that 9 out of 30 (30%) preterm delivered had prior history of preterm labour. Also, in **Chandana et al study** in 2018, 7 women out of 30 women (23.3%) who delivered preterm had history of preterm birth. This is also in accordance to our study where only 18% women with preterm delivery had prior history of preterm birth.

Hence history of preterm birth was not significant in predicting preterm delivery independently, because the significance of genetic risk factors in preterm is heavily reliant on gene-gene and gene-environment interactions, which may or may not.

Table 3: Comparison of Preterm birth history in various studies:

S NO	STUDY	Preterm birth history
1.	2011- Turan et al	29.6%
2.	2015-Guler et al	14%
3.	2015-Ibrahim et al	9.3%
4.	2017-Agarwal et al	30%
5.	2018-Chandana et al	23.3%
6	Our study	18%



In our study, women with a mean enlarged cFAGV of 397 mm³/kg presented with preterm features within 8+/- 3days. cFAGV of 273.04 had 92.31% sensitivity and 95% specificity in predicting development of preterm features whereas 331.04 mm³/kg had 92.31% sensitivity and 83.33% specificity in predicting preterm delivery.

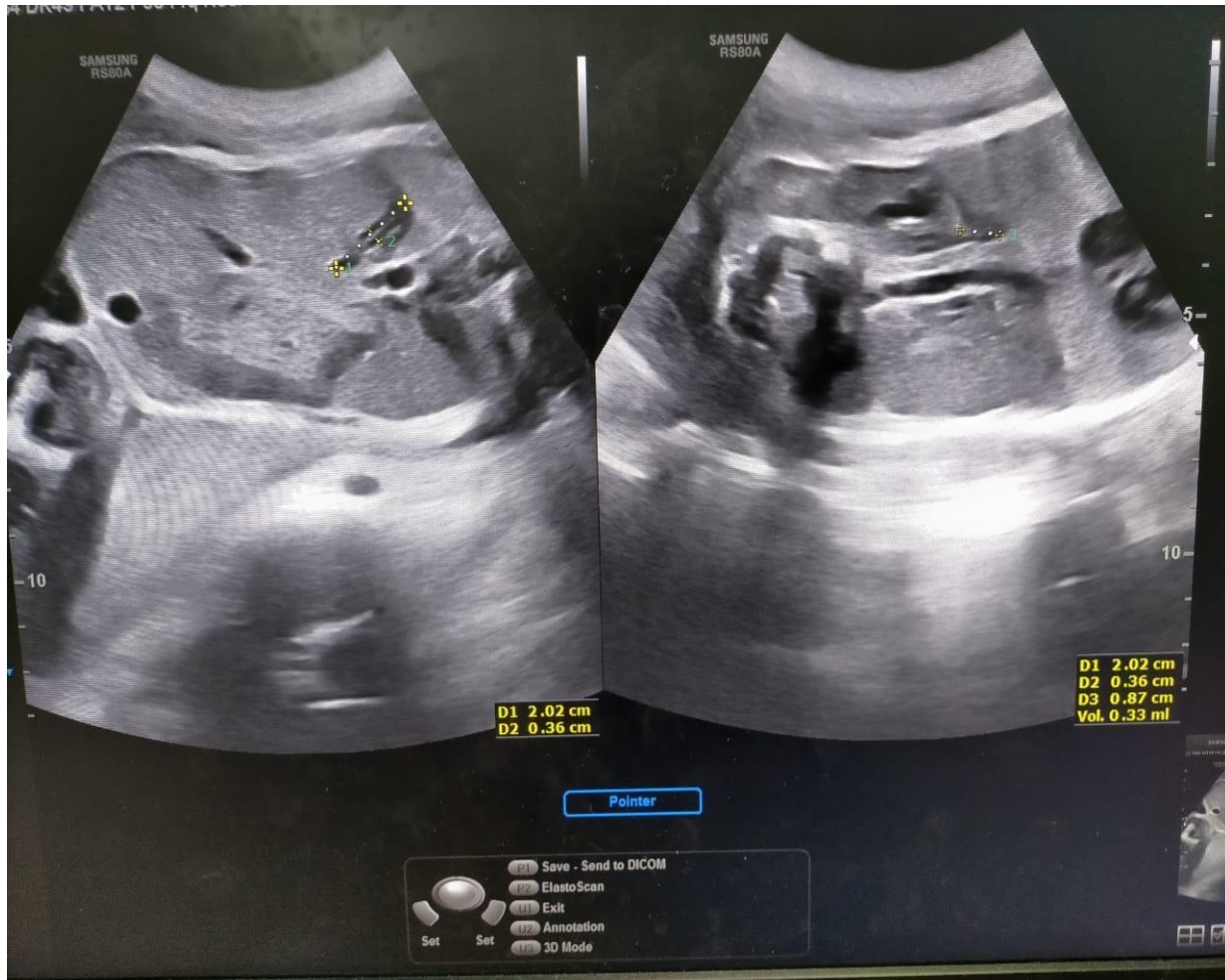
In 2007, Turan et al study, out of 126 women, 53 women were in the preterm group with high risk of preterm delivery including PPROM among whom 23 delivered preterm within 5days. They found a significant cut off of 422 mm³/kg had 92 % sensitivity and 99 % specificity in predicting preterm delivery within 5 days of adrenal gland volume evaluation in both groups. A similar study in 2011 conducted by Turan et al, in a cohort of 74 women within a gestation age of 21 to 34 weeks including PPROM, 27 women delivered within 7days. They found that a cFAGV of >420mm³/kg had 81% sensitivity and 87% specificity in predicting preterm delivery within 7days of ultrasound evaluation of fetal adrenal gland. In a study conducted in 2015 by Ibrahim et al, 75 women with threatened preterm labour were compared with fetal fibronectin ,cervical length, FZE and FAGV. Among them, 27 delivered within 7days of ultrasound scan, 34 delivered after 7days (<37 weeks) and 14 women delivered >37 weeks. This was the first study to exclude PPROM in the study sample to limit the analysis to PTB process alone and to exclude the other inflammatory mechanisms leading to preterm delivery. They found that cFAGV of more than

405 mm³/kg had 92.6% sensitivity and 95.8% specificity in predicting preterm delivery within 7days. Hoffman et al study in 2016, on the other hand, found that none of the fetal adrenal measures were predictive of PTB. It was a study to see if ultrasonography measurements of the fetal adrenal gland taken far away from delivery may reliably predict spontaneous preterm birth in asymptomatic women. A prospective multicenter observational nested cohort study of asymptomatic nulliparous women with a singleton pregnancy between 22 and 30 weeks of gestation was done There were 82 (4.8 percent) spontaneous preterm births at fewer than 37 0/7 weeks of gestation and six (0.4 percent) spontaneous preterm births at less than 34 0/7 weeks of gestation, respectively. There was no difference between spontaneous preterm delivery and term birth based on any of the fetal adrenal gland parameters. The fact that none of their participants delivered within two weeks of ultrasound assessment, and the mean time interval between ultrasound and delivery was 55.3 days, ranging from 18 to 93 days, is attributed to these contradictory findings. *As a result, it supports the idea that the fetal adrenal gland enlarges during a brief period of time, lasting from 7 to 14 days, and that only during this expansion ,it is highly predictive.* According to Agarwal et al -2017, biometry of the fetal adrenal gland and prenatal cervical elastography, are useful in predicting premature delivery. It was a study conducted in 30 women in preterm labour within 28 to 37 weeks period of gestation including PPROM with equal number

of control group of 37weeks gestation. They found that cFAGV of 415 mm³/kg had 67% sensitivity and 76% specificity in predicting preterm delivery. The major drawback was the sample size. A pilot prospective study by **Saber ali et al** was conducted in 2018. The fetal adrenal blood arteries were examined using Doppler ultrasound. For each women, the RI, PI, and S/D ratios were determined. In the end, 30 pregnant women were enrolled in the research. Those who delivered within 7 days (group (I) n=13) and those who delivered 7 days or

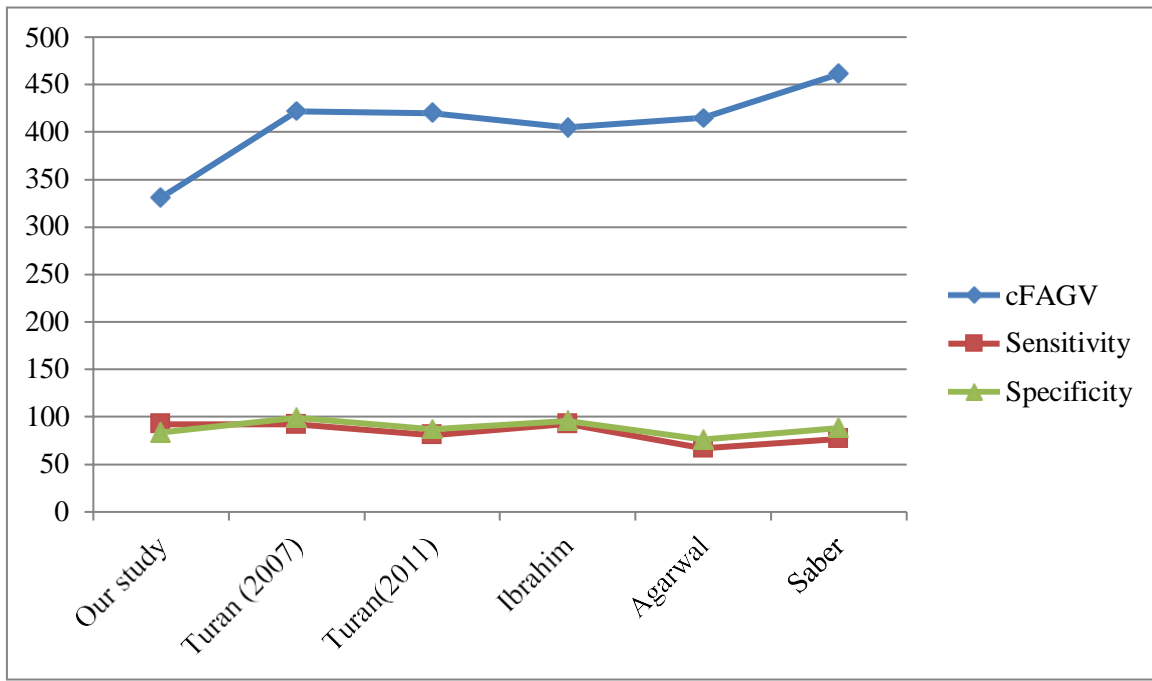
beyond (group (II) n=17) were compared. A cutoff of more than 461mm³/kg had 76.92% sensitivity and 88.24% specificity in predicting preterm delivery within 7days. The authors of this study discovered that preterm delivery within 7 days had an increase in fetal adrenal gland volume, implying a direct positive association between fetal adrenal gland volume and preterm birth within 7 days. These findings support our study which showing an increase in fetal adrenal gland volume at the time of examination for preterm birth symptoms.

IMAGE OF FETAL ADRENAL GLAND:



STUDY	cFAGV	Sensitivity	Specificity
Our study	331	92.31	83.33
Turan (2007)	422	92	99
Turan(2011)	420	81	87
Ibrahim	405	92.6	95.8
Agarwal	415	67	76
Saber	461	76.92	88.24

Table 4: Comparison of cFAGV in various studies.



In our study, we found that fetal zone enlargement was significant in predicting preterm labour. We found that Width ratio(w/W) had 96.88% sensitivity and 79.41% specificity in predicting preterm labour. Depth ratio (d/D) had 96.88% sensitivity and 75% specificity in predicting preterm labour. According to **Turan et al study in 2011**, **depth ratio** was superior among the other factors to predict preterm birth within 7days of its measurement. He found that depth ratio (d/D=0.49) had higher efficacy with 100% sensitive and 89% specific in predicting preterm labour when compared to width ratio (w/W=0.51) with 74% sensitivity and 85% specificity . The rise in fetal zone, successfully stratified the risk of preterm birth .The expansion of the fetal zone indicates an acute activation of the parturition mechanism but in the absence of overt intrauterine infection, predicting latency duration in preterm patients remains a clinical challenge. For predicting delivery, several inflammatory indicators in amniotic fluid were utilised, however these procedures required invasive testing.(83,84) All individuals in their PPRM group who had an expanded fetal zone delivered within 7 days. If the fetal zone was within normal bounds, the latency period extended to 14 weeks. At best, the role of tocolytic therapy in extending pregnancy is debatable. Despite extensive study, practitioners are unable to determine which patients may benefit from treatment. In their high-risk group, they discovered that if the fetal zone is increased , despite tocolytic therapy, patients deliver within 7 days. **These findings show that the FZE's predictive value is independent of the clinical conditions that are linked to**

PTB. Guler et al study which was conducted in 2015 to compare preterm with term labour in terms of depth ratio(d/D). As a comparison group, two evaluation groups were developed, that includes 29 (46.7%) preterm patients and 33 (53.3%) term cases. The **FZD/TGD ratio** was statistically considerably higher in preterm babies than in term births (55.4 percent 4.9 vs. 47.7% 5.6) (P 0.001). Though they appear to be comparable in terms of mechanism and fetal reaction, preterm and term labours are really quite different, especially when it comes to anatomical responses to hormonal effects such as an increase in the depth of the fetal adrenal gland. The preterm birth group had a greater FZD/TGD ratio than the term birth group (P 0.001). When all risk factors for preterm labour are considered, all of them contribute to a challenging intrauterine fetal growth and development environment. While this environment demands a high stress adaptation mechanism, the lack of such traits in a term fetus explains the difference between the two comparison groups. The findings of their study suggest that ultrasonographic evaluation of the fetal adrenal gland and fetal zone enlargement may be advantageous in situations of preterm labour symptoms. **In 2015, Ibrahim et al study cFAGV and FZE (width ratio = w/W)** were the only independent significant predictors of delivery within 7 days among the examined patients after controlling for other factors in a multivariate logistic regression analysis. In their study, conducted in threatened preterm women, width ratio (fetal zone enlargement) had higher efficacy when compared to

depth ratio. Width ratio ($w/W=0.47$) had 92.6% sensitivity and 89.6% specificity in predicting preterm delivery within 7 days, whereas Depth ratio ($d/D=0.52$) had only 70.4% and 70.8% sensitivity and specificity respectively. The limitations of this study include the lack of a group of pregnant women at a low risk of PTB and the use of qualitative fetal fibronectin assay. The current study, being a cohort study, did not include a control group of healthy asymptomatic pregnant women. The studied cohort was stratified according to the occurrence of PTB within 7 days into two groups; those who did not deliver within 7 days of enrollment were considered as study controls. They suggested that further larger studies are recommended to see if cAGV and FZE could predict PTB in the low-risk population. Finally, **they concluded that, in women presenting with threatened PTL in the third trimester of pregnancy, cAGV and FZE could be used as independent predictors of delivery within 7 days.**

According to a study conducted in 2017 by Agarwal et al, when he compared preterm with term, fetal zone enlargement which they considered as fetal zone depth ratio (d/D) and shear wave speed estimation of antenatal cervix was highly predictive of preterm labour. FZE and cFAGV revealed greater values in the preterm group than the term group, with statistical significance, FZE and cAGV. In this study, fetal zone enlargement ($d/D=0.47$) had 90% sensitivity and 80% specificity in predicting preterm delivery. Unlike the above study, **Width ratio was superior in our study with**

higher specificity than depth ratio. Similar to ours, in Chandana et al study, in 2018, they found that **width ratio (w/W)** had the best efficacy when compared to depth ratio followed by cFAGV in estimating preterm delivery. Width ratio ($w/W=0.62$) had 96.67% sensitivity and 86.2% specificity when compared to depth ratio ($d/D=0.55$) which had 80% sensitivity and 54% specificity in predicting preterm labour. **Goletzke et al study** was conducted in 2020. In a low-risk population, longitudinal growth evaluations of the fetal adrenal gland were performed, with a special focus on trajectories in preterm fetuses. Fetal adrenal gland was examined through transabdominal ultrasonography at gestational weeks (gw) 24–26, 28–30, and 34–36 in a low-risk pregnant cohort. The total gland and the mark (so-called fetus zone) longitudinal trajectories, as well as the ratio of fetal zone width to total widths (w/W), were studied. The width of the fetal zone expanded during pregnancy ($p < 0.0001$), but the ratio w/W decreased ($p < 0.0001$) ($n = 327$) as the gestational age advanced. When the **trajectories of the ratio w/W in preterm fetuses** ($n = 11$) were compared to propensity-score matched term born fetuses ($n = 22$), both groups showed a reduction between gw 24–26 and 28–30, which **continued to decline for the term born fetuses. Preterm born fetuses, on the other hand, had a higher ratio than term born fetuses at weeks 34–**

36. The study provides the first longitudinal growth data on the fetal adrenal gland and backs up the theory that fetal zone enlargement is linked to preterm delivery, which might be useful in risk prediction,

Table 5 :Comparison of width ratio in various studies:

S.No	STUDY	Width ratio(w/W)	Sensitivity	Specificity
1.	Our study	0.54	96.88	79.41
2	Chandana et al	0.62	96.67	86.2
3	Turan et al	0.51	75	84
4	Ibrahim et al	0.47	92.6	89.6

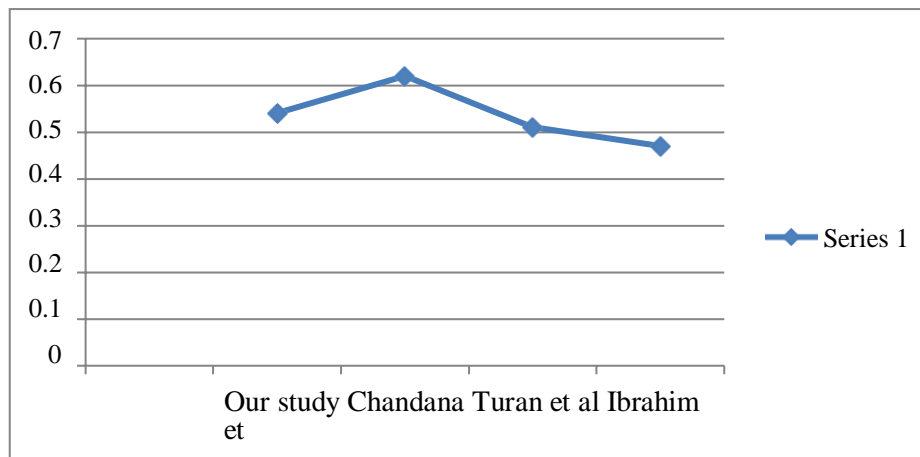
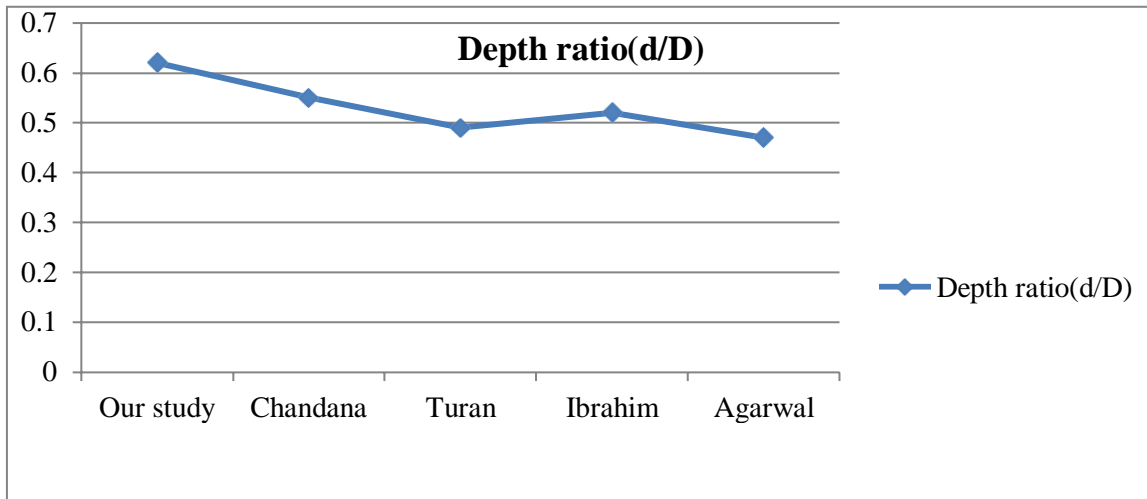


Table 6: Comparison of Depth ratio in various studies:

S.No	STUDY	Depth ratio(d/D)	Sensitivity	Specificity
1.	Our study	0.62	95.83	66.67
2.	Chandana	0.55	80	54
3.	Turan	0.49	100	89
4.	Ibrahim	0.52	70.4	70.8
5.	Agarwal	0.47	90	80

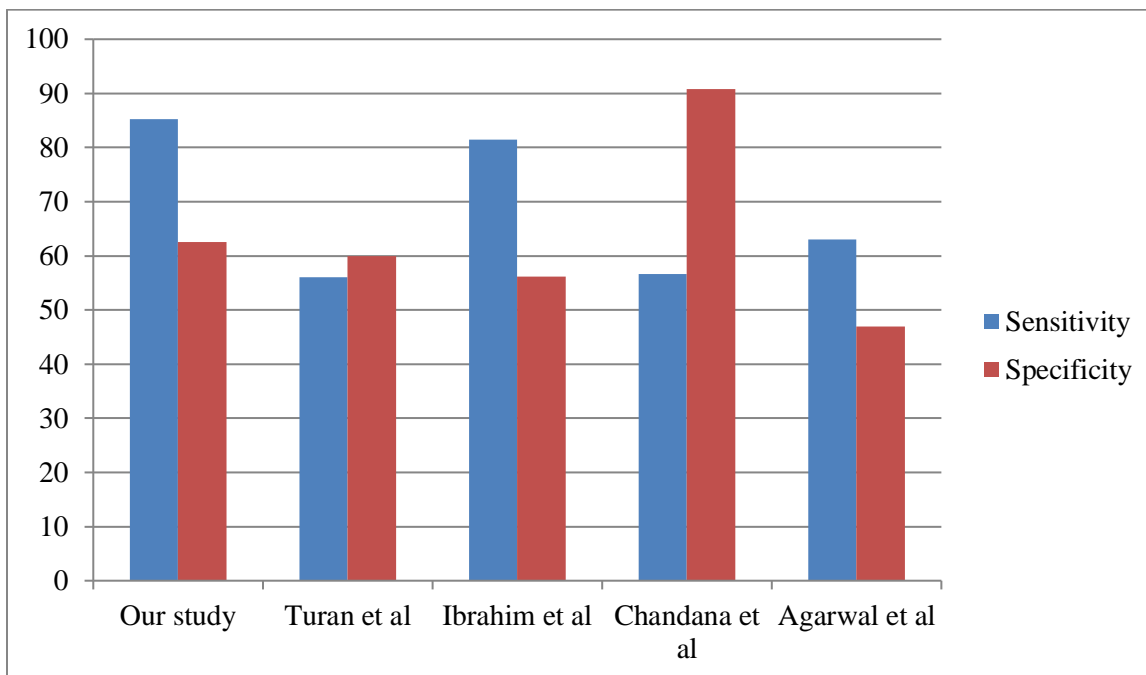


In our study, cervical length had 85.2% sensitivity and 62.5% specificity in predicting preterm labour. In 2011, Turan et al study, Cervical length had no correlation in those who delivered within or after 7 days. Hence, measurement to delivery interval was significant for cAGV and FZE whereas Cervical length was not significant. These results matched those of a previous Cochrane systematic review. (85) The research concluded that there is inadequate evidence to warrant regular CL measurement screening of asymptomatic or symptomatic pregnant women. This is in acceptance to this study where cervical length sensitivity and specificity were 56% and 60%. In Guler et al study in 2015, cervical length was not found to be significant to detect preterm delivery (p=0.27). In 2015,

Ibrahim study, cervical length had 81.5% sensitivity and 56.2% specificity in predicting preterm labour within 7 days of its assessment. In 2018, Chandana et al study, cervical length had reduced efficacy when compared to fetal adrenal gland parameters. It had 56.67% sensitivity and 90.8% specificity in predicting preterm labour if less than 2.5cm. In 2017, Agarwal et al study, cervical length of 2cm had only 63% sensitivity and 47% specificity in those who went into preterm labour *whereas cervical elastography had the highest efficacy* of 96.7% sensitivity and 87% specificity in predicting preterm labour. **Hence cervical length has reduced efficacy in predicting preterm labour if compared to fetal adrenal gland parameter.**

Table 7: Comparison of cervical length in various studies:

S.No	STUDY	Sensitivity	Specificity
1.	Our study	85.2	62.5
2.	Turan et al	56	60
3.	Ibrahim et al	81.5	56.2
4.	Chandana et al	56.67	90.8
5.	Agarwal et al	63	47



In our study, 78 women progressed till term delivery among whom 13 had to be induced and the rest 55 delivered spontaneously. Among the spontaneously delivered women, mean cFAGV was 384mm³/kg when compared to 277mm³/kg in induced women. A cutoff of 361mm³/kg shows 87.5% sensitivity and 91.67% specificity in predicting spontaneous onset of labour. Width ratio (w/W=0.65) shows 77.08% sensitivity and 83.33% specificity, depth ratio(d/D=0.62) showed 95.83% sensitivity and 66.67% specificity in predicting spontaneous onset of labour.

In 2018, Chandana et al study, a mean fetal adrenal gland volume of 393.05 mm³/kg was found in spontaneously delivered term women when compared to 290.92 mm³/kg in induced women. At the scan done at term, they discovered a statistically significant difference in the cFAGV between these two groups (spontaneous labour 393.05 mm³/kg versus forced labour 290.92 mm³/kg; p value 0.01) similar to our study. **Mathew and Alexis et al 2019 study** was a prospective cohort

research that looked at the ability of 2- dimensional ultrasound measurements of fetal adrenal gland total length, total breadth, fetal zone length, and fetal zone width to predict the beginning of spontaneous term labour in women in their *third trimester*. The trial was completed by 40 patients who satisfied the inclusion criteria. 11 (27.5%) of the individuals were in spontaneous labour, whereas 29 (72.5%) were induced into labour. This study is a diagnostic test accuracy study that uses a prospective cohort design to show that ultrasonographic fetal adrenal gland measures may predict spontaneous term labour relatively well. Their main finding was that an ultrasound measurement *of fetal w/W cut-off point of 0.41* predicted spontaneous term labour with high sensitivity (91.0%) but poor specificity (44.8%), indicating that this is an useful screening test for term spontaneous labour. In this small prospective cohort, ultrasound-measured fetal w/W was shown to be marginally predictive with spontaneous labour. Limitation of this study was the sample size.

	cFAGV in Spontaneous (mean)	cFAGV in Induced (mean)
Our study	361.74	277.45
Chandana et al	393.05	290.92

Table 8: cGAFV in spontaneous and induced labour

STRENGTH:

The strength of our study was to examine the aforementioned factors in women who only had

threatening PTL after removing women with PPROM from the final statistical analysis. Women with PPROM were included in studies that examined the diagnostic

accuracies of 2D ultrasound assessments of cAGV and FZE as predictors of PTB to CL [8–10]. When compared to spontaneous intact membrane PTB, PPRM has been linked to a higher incidence of inflammation, suggesting that the underlying processes for both occurrences are distinct.

LIMITATIONS:

This study is done in limited number of subjects due to COVID pandemic. These results may vary if done in a large number of subjects.

CONCLUSION:

In a prospective study conducted in KGH, with a 100 sample size, majority of patients were primigravida, less than 25yrs, a significant correlation was found between increased fetal adrenal gland and fetal adrenal zone enlargement in those who delivered preterm. Enlarged fetal adrenal gland volume of 331 mm³/kg and enlarged fetal width ratio are an excellent predictor of preterm delivery in this study when compared to depth ratio and cervical length. Also this study found that fetal adrenal gland volume enlargement can predict the occurrence of spontaneous labour. On followup scan at term gestation of 37 to 39 weeks, spontaneous labour group had more adrenal gland volume when compared to those who had to be induced.

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