ABSTRACT
Amniotic fluid envelops the fetus during the gestation period. The abnormal high fluid volume (polyhydramnios) can alert fetal anomalies. The inadequate amount of liquid (oligohydramnios) causes poor lung development. Mild Polyhydramnios occurs in 17% of pregnancies whereas oligohydramnios occurs in 4.5% of pregnancies and severe oligohydramnios occurs in 0.7% of all pregnancies out of which, only 16.5% is diagnosed and the fetal mortality rate is high if not properly treated. This review deals with the periodic monitoring of amniotic fluid in the amniotic sac during the third trimester as the incidence of oligohydramnios is high during that period. The growth of the uterus stops at 5 months and the uterus expands due to the internal pressure exerted by the fetus and amniotic fluid. The amniotic fluid pressure is proportional to the uterine contraction and inversely proportional to the fluid volume. The radius of the amniotic sac pocket decreases as the fluid volume decreases which can cause an increase in uterine contraction.

Keywords
Amniotic fluid, amniotic fluid pressure, fetus, oligohydramnios, polyhydramnios.

1. INTRODUCTION
The amniotic sac forms about 12 days after conception and the amniotic membrane consists of two layers namely the amnion and the chorion. The maternal plasma generates the amniotic fluid and it passes through the fetal skin by osmotic and hydrostatic forces. The amniotic membrane thickness varies from 0.72 mm to 1.08 mm. The anterior abdominal wall consists of muscle (strength) layer, fatty layer (Camper’s fascia & Scarpa’s fascia) and skin. The muscle layer consists of rectus abdominalis muscle and aponeuroses of external oblique, internal oblique and transversus abdominis muscle. The posterior abdominal wall consists of lumbar vertebrae and intervertebral discs. The thickness of rectus abdominalis varies from 9.60 mm in the xiphoid region to 10.26 mm in the umbilical region. The thickness of subcutaneous fat layer varies from 23.39 mm to 24.31 mm. The thickness of the abdominal muscle reduces in pregnancy and gets separated as the baby grows. The aneuploidies and chromosomal abnormalities (structural) can be identified on culturing the amniotic fluid at 12-14 days. Many techniques have been evolved since this culture method is time consuming. The methodologies followed in detection of chromosomal abnormalities in fetus includes Fluorescence In Situ Hybridization (FISH), Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR), Multiplex Ligation-dependent probe Amplification (MLA), Bacs-on-beards microarrays, microarrays and non-invasive prenatal diagnosis. [1]
**Composition of fluid**

Amniotic fluid initially consists of water from mother and later baby’s urine makes up the fluid. It also consists of nutrients, hormones and antibodies. The nutrients include proteins, lactate, pyruvate, peptides, carbohydrates, lipids, phospholipids, urea and electrolytes. The hormones include Prolactin, Growth hormone, Placental hormone, Human chorionic gonadotropin, Human chorionic somatomammotropin, Human chorionic corticotropin, Human chorionic thyrotropin and placental luteinizing hormone-releasing factor. The biochemical products, nutrients and water are interchanged between the mother and fetus. The biochemical products facilitate renal maturation of fetus. The determination of acetyl cholinesterase and butyrylcholinesterase in the fluid is very important. The acetyl cholinesterase hydrolyses the neurotransmitter into choline and acetate. The acetyl cholinesterase is present in the synapses of cholinergic synapse and neuromuscular synapse. The determination of these enzymes can be done by the Choline sensor.

**Significance of amniotic fluid**

The amniotic fluid protects the fetus from mechanical jerks and shocks. It promotes skeletal and muscular development. It helps in the development of lungs and formation of gastrointestinal tract. MSC sources are Neonatal tissues such as umbilical cord, placenta, and amniotic membrane. Amniotic fluid is collected during caesarean section (C-section) deliveries using a closed catheter-based system. After the fluid processing, amniotic fluid is assessed for cellularity, MSC (Mesenchymal stromal cells) frequency, in-vitro proliferation, surface phenotype, differentiation, and gene expression characteristics. The Mesenchymal cells are used in disease modeling, pharmaceutical screening and regenerative medicine by reprogramming technologies. The pluripotent stem cells showed multigerm layer lineage differentiation potential. The breathing activity of the fetus helps to eliminate the amniotic fluid. The fetus swallows the amniotic fluid about 200 - 250 ml/day approximately.

**Fluid volume**

The fluid level increases from 10-20 ml at initial phase to 1000 ml at 38 weeks and decreases to 800 ml towards the delivery. The rate of change of fluid varies from 10 ml/week (initial phase) to 60 ml/week at 21 weeks and decreases at a rate of 8% per week in the third trimester. The fetus secretes the fluid from oral, nasal, tracheal passages and it ranges from approximately 60 – 100 ml/kg. Underproduction of fluid is due to absence or dysfunctional kidneys, urinary tract obstruction, abnormal placental function and maternal dehydration. Further, fluid Loss is due to the rupture of the amniotic membranes (ROM).

**Conditions of amniotic fluid**

An AFI < 5-6 cm is considered as oligohydramnios. It leads to limb contractures, clubbing of feet and hands, hypo plastic lungs (alveolar spaces are not completely developed). The causes includes obstruction of urinary tract, cystic dysplasia, Meckel Gruber Syndrome, central nervous system malformation, pulmonary hypoplasia, VACTERL association, chromosomal factors, congenital factors, intrauterine growth restriction, post-term pregnancy, premature ROM, fetal demise, placental abruption, twin to twin transfusion, maternal dehydration, uteroplacental insufficiency, hypertension, pre-eclampsia and chronic hypoxia. Intrauterine Growth Restriction (IUGR) is associated with oligohydramnios.

An AFI > 20-24 cm is considered as polyhydramnios. It causes fetal malformations, maternal diabetes mellitus, multiple pregnancies, fetal anemia, viral infections, Barter syndrome, neuromuscular disorders, and maternal hypercalcemia. Infections include parvovirus B19, rubella, cytomegalovirus, toxoplasmosis and syphilis.
Analysis of Amniotic Fluid

The analysis of amniotic fluid reveals the uterine infections, fetal genetic health, Rh incompatibility, fetal lung maturity, age and viability of the fetus. Amniocentesis is an analytic procedure, involves injection of a long needle into the amniotic sac and the fluid is collected (less than one ounce) under the guidance of ultrasound. It indicates Sickle Cell disease and genetic disorders like Down’s syndrome, Cystic fibrosis, etc. The maternal fluid testing reveals the presence of Congenital Toxoplasmosis. The amniotic fluid collected at 20-28 weeks can indicate the presence of ZIKV RNA (ziga virus) in microcephalic cases. The gut colonization in fetus is associated with the microbial infection in amniotic fluid and meconium. The microbial advent continues even after delivery from mother to infant through breast milk.

![Figure 1: amniotic fluid variation with gestational age](image)

Fluid dynamics

The maternal plasma and fetal plasma are in equal proportion for 4 months. The protein concentration decreases in the maternal plasma. The amniotic fluid surrounding the fetus is regulated by four main factors such as fetal urine production, lung liquid secretion, swallowing and intramembranous absorption. The active and passive components are present in the amniotic fluid. The fluid regulation is mainly carried out by absorption through amniotic epithelial cells.

![Figure 2: amniotic fluid regulation](image)

The fluid produced by the fetus per day is sufficient to replace the entire amniotic fluid for every 12-24 hours. The deviations in the amniotic volume can lead to perinatal morbidity and mortality. Fetal Growth Restriction (FGR) is considered as the second most common cause for perinatal morbidity. The fluid regulation is modulated by the stimulators and inhibitors present in the fluid and its surroundings. The stimulators are present in the fetal urine and the inhibitors are secreted by the amniotic membranes. The active components are mostly modulated rather than the passive components.

Osmolality refers to the amount of chemicals dissolved in the blood serum. It is said that the Osmolality of the amniotic fluid and the maternal plasma are same. The amniotic fluid is a transudate of maternal plasma across the fetal skin or the placental surface. The fetal skin is non-keratinized till 22-25 weeks for the effective passage of the fluid across it. After 24 weeks, the
amniotic fluid is exchanged through the nose and mouth of the fetus but not to a greater extent. The fetal renal system acts as the major source for amniotic fluid. The kidneys start to function at 8-11 weeks of gestation. The fetal urine output varies between 7-70 ml/hour from 24 weeks. Fetal respiratory activity begins at 11th week. The fluid is also excreted by the fetal pulmonary system. The half of the secreted lung fluid enters the amniotic fluid while the other half is swallowed. The production and reabsorption of the amniotic fluid is maintained in equilibrium.

II METHODOLOGY

Normal volume can be estimated in two ways. It can be mathematically estimated using area under the curve in a distributed population. The lower and upper 5% are excluded as abnormal. It can also be defined in terms of outcome in which undesired outcomes and measurements are defined as abnormal. Oligohydramnios was poorly identified with 3rd and 5th percentiles but neither one was superior to the other. The amniotic fluid volume is less than 200 ml or 500 ml. The Single Deepest Pocket (SDP) is less than 2 cm, Amniotic Fluid Index (AFI) is less than 5 cm or it is below the 5 th percentile for gestational age. The amniotic fluid can be measured directly, indirectly or estimated sonographically. Direct measurement is done during the times of cesarean or uterine hysterectomy. Indirect measurement is done by dyes dilution techniques. Non-invasive screening can be done by MRI but cannot be done for everyday screening.

There are four methods of sonographic evaluation of amniotic volume namely, subjective assessment, 2x2 measurement, single deepest pocket(SDP) or maximum vertical pocket(MVP) and amniotic fluid index(AFI). Amniotic fluid index is obtained by the summation of the vertical diameter of the largest pocket in each of the four quadrants with the maternal umbilicus as the reference point. The transducer used for measurement should be placed in a longitudinal plane and a minimum horizontal measurement of one centimeter for each pocket should be maintained. The amniotic sac diameter can be used to find out the weight of the fetus. The sonography measurement of the gestational sac, Mean Sac Diameter (MSD) is calculated at around three weeks after gestation which ranges about 2-3 mm.

\[
MSD = \frac{\text{length} + \text{height} + \text{width}}{3}
\]

Single Deepest Pocket is found by identifying the largest pocket of the amniotic fluid by a global assessment of all pockets and selecting the largest vertical measurement with a minimum horizontal measurement of one centimeter.

<table>
<thead>
<tr>
<th>Table 1: Mean values of AFI</th>
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<tbody>
<tr>
<td>Week</td>
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</tr>
<tr>
<td>34</td>
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<tr>
<td>35</td>
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<td>36</td>
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<tr>
<td>37</td>
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<tr>
<td>38</td>
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<tr>
<td>39</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: The normal and median level of AFI</th>
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<tbody>
<tr>
<td>Condition</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>Median</td>
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</table>
A two diameter pocket is calculated by multiplying the depth and width of the largest single pocket.

Amniotic fluid assessment
The perinatal risks can be found by assessment of the amniotic fluid. The mostly used technique is sonography while accurate techniques are Amniotic Fluid Index (AFI) and Maximum Vertical Pocket (MVP). MVP should always be considered for diagnosing AF abnormality as AFI sometimes overdiagnoses the abnormalities which can lead to increased rate of pregnancy intervention. The more accurate technique is dye dilution, an invasive technique and can be performed only in caesarean sections. The color Doppler is used to identify the umbilical cord in amniotic fluid for oligohydramnios diagnosis but it has labeled 37% of women with normal AFV as oligohydramnios. 3D ultrasonography can be used to assess third trimester AFV since the AFV in third trimester is proportionate value of fetal urine production and its normal range indicates good placental perfusion and fetal nutrient and oxygen transfer. The determination of AFI in the last trimester becomes a standard for antenatal care. The magnitude of AFI varies at weekly intervals hence it is recommended to carry out the ultrasound examination for every two weeks after 34 weeks in case of low risk pregnant women.

Amniotic fluid leakage
The membrane surrounding the fetus ruptures and this leads to leakage of amniotic fluid. This increases the risk of bacterial infection and affects both maternal and fetal health. Oligohydramnios in the midtrimester following PPROM will cause a delay in fetal lung development (pulmonary hypoplasia). The swallowed amniotic fluid makes up the formation of meconium which is the first stool of the infant that contains the bile, water and amniotic fluid digested by the fetus. Repeated trans abdominal amnioinfusion is performed in which an isotonic solution is filled in the amniotic cavity to alleviate oligohydramnios and thicken the meconium.

Vision Amniotic Leak Detector (ALD) can detect amniotic fluid leakage as a cause of vaginal wetness. Alpha feta protein (AFP) is a glycoprotein that is produced in early life of the fetus (fetal liver) and it is elevated at the time of pregnancy. AFP test is a one-step self-test to detect amniotic fluid leakage, AFP levels can assist in differentiating amniotic fluid from other bodily fluids. If the vaginal discharge is associated with AFP, then the membranes have been ruptured. Alpha-fetoprotein levels in amniotic fluid (245.38±21.03 ng/ml, n=52) were significantly higher in maternal urine (0.84±0.17 ng/ml, n=52, P<.001), semen (1.52±0.35 ng/ml, n=17, P<.001). The diagnosis is combined with evaluation of vaginal fluid and nitrazine for evidence of ferning or with monoclonal antibodies to detect placental alpha-microglobulin-1 (PAMG-1). [8]

Labor cases
The amniotic cell epithelial to Mesenchymal transition [EMT] is closely associated with labor. The EMT decreases the tensile strength of the amnion membrane. The biochemical weakening of amnion membrane can lead to preterm labor due to premature rupture of membrane [PPROM]. In prelabor, the cervix is thinned and undergoes gradual dilation. The pregnant women must be admitted to the hospital before the cervix widens to 4cm. The first stage of labor usually lasts from few hours to 12 hours. If the dilation maximizes to10cm, the pushing action is required. In case of silent labor; the cervix contraction may fail even after the delivery of the fetus.

III RELATION BETWEEN FETUS AND AMNIOTIC FLUID VOLUME
As the myometrial tension increases, the uterine contraction reduces. The amniotic pressure during-trimester is greater than the pressure in full term. This is due to the size of the uterus which is smaller in the mid-trimester than in the full term. The ratio of amniotic fluid volume to the fetal volume increases till 30 weeks and starts to decline towards the delivery. The insufficient quantity of amniotic fluid can cause contact between the fetal parts and the uterine wall and this isolates the pockets of the amniotic fluid.

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The fetal movement gradually increases in the second trimester and decreases at term. The fetal movements can be used to determine the health status. It is found to be 74 times per hour at 28 weeks and 29 times per hour at term. The fetus is immobile during sleep. Steigman SA, et al determined the optical properties of the amniotic fluid for optimizing the video image processing during the video fetoscopy. The light transmission in the amniotic fluid decreases throughout the gestation. The peak refraction of the amniotic fluid ranges within a relative window of near infrared spectrum at 848.1 +/− 52.3 nm. Light reflection is negligible in all the amniotic fluid samples. [9]

<table>
<thead>
<tr>
<th>Ultrasound estimation</th>
<th>Oligohydramnios</th>
<th>Normal values</th>
<th>Polyhydramnios</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFI</td>
<td>&lt;5 cm</td>
<td>5-25 cm</td>
<td>&gt;25 cm</td>
</tr>
<tr>
<td>SDP</td>
<td>&lt;2 cm</td>
<td>2-8 cm</td>
<td>&gt;8 cm</td>
</tr>
<tr>
<td>2x² diameter pocket</td>
<td>&lt;4 cm²</td>
<td>4-50 cm²</td>
<td>&gt;50 cm²</td>
</tr>
</tbody>
</table>

Table 3: comparison of the amniotic fluid measurement techniques

Anatomical structures of fetus
Biophysical profile is the ultrasound estimation (scoring method) of the fetus which measures five factors namely, fetal heart rate, fetal breathing, fetal movement, fetal tone, amniotic fluid volume. This test is preferred when the non-stress test shows no improvement or less reactive. The sleep and wake patterns for every fetus is unique and the fetal movements reduces at term. The response to external sound and touch is high in the third trimester than in the early phase of pregnancy. The fetal response is selective to the maternal touch and speech. Deep learning based method can be used to segment the amniotic fluid and the fetal tissues from the ultrasound images by using encoder-decoder network. The input image is encoded into down scaled feature maps using convolution techniques and pooling structures. The down scaled structures are up-scaled by using unpooling and convolution layers. The additional convolution layers are used to improve the model and the weights are updated by fine tuning of the pre-trained model.[10]

The Developmental Instability (DI) can be indirectly measured by Fluctuating Asymmetry (FA). The limbs of the deceased fetus will have increased asymmetry since there is insufficient amniotic fluid and increased mechanical pressure. The urogenital abnormality directly affects the amniotic fluid volume. Both the mechanical pressure and the urogenital abnormalities increase the FI. But the effects of mechanical effects are less considered than urogenital abnormalities.[11]

Somatosensory cortex, present in parietal lobe of brain receives all the sensory inputs from the body and processes these sensory signals. Somatosensory cortex is not fully developed without the presence of amniotic fluid. The fluid resistance provided by the amniotic fluid is responsible for the development of the somatosensory cortex of the fetus in uterine environment.

The amniotic fluid comprises of tropic factors and nutrients in order to nurture gastrointestinal tract. The gastrointestinal tract of the fetus develops and its growth is supported by genetic preprogramming,
endocrine secretion (local & systemic) and the swallowed amniotic fluid. [12]

Table 4: Fetal growth

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Development of organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Heart starts to beat</td>
</tr>
<tr>
<td>8</td>
<td>Breathing tubes extend into lungs</td>
</tr>
<tr>
<td>14</td>
<td>Kidneys starts functioning</td>
</tr>
<tr>
<td>26</td>
<td>Inhalation and exhalation of amniotic fluid</td>
</tr>
<tr>
<td>35</td>
<td>Complete development of kidney</td>
</tr>
<tr>
<td>37</td>
<td>Complete development of lungs</td>
</tr>
<tr>
<td>39</td>
<td>Full term</td>
</tr>
</tbody>
</table>

Amniotic fluid embolism

The variations in the fluid volume could lead to amniotic fluid embolism in which the fetal material (amniotic fluid and meconium) is circulated in the blood stream of the mother. The progression of Amniotic fluid embolism (AFE) in amniotic fluid is analyzed using the level of fibrinogen present. Amniotic fluid embolism can be clinically diagnosed based on the four symptoms namely cardiovascular collapse, respiratory distress, coagulopathy, coma/seizures. Among which, coagulopathy is considered to be the main symptom for AFE. Autopsy is the only available laboratory method to find out the presence of the fetal material in the maternal circulation.

The immunological response for Amniotic fluid Embolism is given by the mother to the fetus. Two immunological responses namely Anaphylaxis and Complement activation in AFE have been accounted. These responses can be used only as investigational tools and cannot be used as markers for AFE. The amniotic fluid embolism is less diagnosed due to the less availability of the laboratory markers. [13] The amniotic fluid embolism can be detected using a fluorescence detector (dual-gate photosensitive thin-film transistor - DQPTFT). The biomarker detected using this method is Zinc Coprophyrin-I (Zncp-I). The detector used has high sensitivity, low noise output and its detection limit is 35 nmol/L. The fluorescence emission peak is 580 nm when examined with 405 light emissions. [14]

Relationship of Intra Uterine Pressure (IUP) with Oxytocin-augmented labor

Oxytocin-augmented labor is the method of inducing labor by giving syntocinon and pitocin drip and its dosage is increased until the uterine contractions are in a normal pattern as it occurs in natural contractions. The use of intrauterine pressure catheter does not improve the labor outcome than external monitoring. The IUP was measured in Montevideo (MU) units. The labor stage is gradually increased by Oxytocin. Two analyses were carried out with the IUP measurements.

1. First analysis was done with assessed labor outcome in relation with the high intrauterine pressure measured at any stage of pregnancy.
2. Second analysis was done with assessed labor outcome in relation with intrauterine pressure measured at the last vaginal measurement at the first stage of labor.

The women with low IUP have longer gestational age, longer labor and neonates with high birth weight and will have high risk in caesarean sections. IUP measurements were not associated with the neonatal
outcomes. The uterine contraction can be monitored using an external tocodynamometry and intrauterine pressure catheter (IUPC). IUPC associated only with the uterine contraction and it does not have any impact on perinatal outcomes. IUPC inserted with Oxytocin causes blood loss and leads to Cardiotocography of fetus. IUPC creates risk in the fetal well-being.

Table 5: Likelihood ratio for caesarean section in women with High IUP vs. Low IUP

<table>
<thead>
<tr>
<th>IUP (MU)</th>
<th>Likelihood ratio</th>
</tr>
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<tbody>
<tr>
<td>&lt;100</td>
<td>1.6</td>
</tr>
<tr>
<td>&gt;300</td>
<td>0.41</td>
</tr>
</tbody>
</table>

IV CONCLUSION

Amniotic fluid is considered to be an important factor for determining the fetal biophysical profile. Out of all the available techniques, AFI and MVP provide better results in diagnosing the amniotic fluid abnormalities. The amniotic fluid is necessary for fetal growth and organogenesis. The oligohydramnios is strongly associated with gestational age and mortality whereas the polyhydramnios is associated with the birth percentile < 90%. The prediction ability of perinatal outcome is very poor.

References

1. T.F. Ashavad, Laboratory medicine in India, an issue of clinics in laboratory medicine, 2012.
14. Carlo Janzen, Suvaieh Sen, Margarida Y L Leu, Marina Gagliardi de Assumpcao, John Challis, Gaetan Chaudari, The role of epithelial to Mesenchymal transition in human amniotic
30. Ryosuke SASAKI, Yasunori Yamada, Yuki Tsukahara, Yasuo Kuniyoshi, Tactile stimuli from amniotic fluid guides the development of Somatosensory cortex with hierarchical structure using human fetus simulation, IEEE 2013.
34. Viola Marx, Emese Nagy, Fetal behaviour responses to maternal voice and touch, PLOS ONE, 2015.