

The Yolk Sac Abnormalities, Maternal Serum Level of Cancer Antigen 125 (CA-125) and Beta Human Chorionic Gonadotropin (B-HCG) as an Early Predictors of First Trimester Pregnancy Loss in Patients with Threatened Miscarriage: Review Article

Eman Adel Bastawi^{*1}, Mohamed M. Shaaban², Eman Ahmed Keshk^{1,2}, Asmaa M. Elgedawy¹

¹Department of Obstetrics and Gynecology, Faculty of medicine, Suez Canal University, Suez, Egypt

²Department of Obstetrics and Gynecology, Faculty of Medicine, Suez Canal University, Suez, Egypt

*Corresponding Author: Eman Adel Bastawi, Mobile: 01024455657,

Email: pgs.000934406@med.suez.edu.eg

ABSTRACT

Background: Miscarriage is defined as the loss of a pregnancy before to the twenty-week mark or of a foetus weighing fewer than five hundred grams prior to viability. In between 15–25% of pregnancies, the mother has a threatened miscarriage. It is associated with anxiety in the mother, family, and the physicians. More than 50% of pregnancies with threatened abortions end in pregnancy loss. Its fate can be predicted using a plethora of biological markers. However, there is currently no convincing biochemical marker utilized before sonographic diagnosis is confirmed, which may take several weeks to distinct between normal and abnormal pregnancy.

Objective: This article aimed to determine how accurately cancer antigen 125 (CA-125) as well as beta HCG levels in the mother's blood may predict a miscarriage.

Methods: We searched Science Direct, Google Scholar as well as PubMed for relevant articles on Cancer Antigen 125 (CA-125), Beta Human Chorionic Gonadotropin (B-HCG), First Trimester Pregnancy Loss and Threatened Miscarriage. Only the most recent study was taken into account between 1988 and 2022. Documents written in languages other than English have been ignored due to a lack of translation funds. Unpublished works, oral presentations, conference abstracts, and dissertations were generally agreed upon not to qualify as scientific research.

Conclusion: Blood CA 125 levels are a sensitive as well as specific predictor of loss of pregnancy in cases of threatened abortion because of their low cost, ease of availability, as well as lack of inconvenience

Keywords: CA-125, B-HCG, Yolk Sac, Threatened Miscarriage.

INTRODUCTION

Spontaneous abortions are among the most prevalent complications during pregnancy. Currently, 9 hormonal techniques [estradiol (E2), estriol, serum human chorionic gonadotropin, estrone urine human chorionic gonadotropin, progesterone, cortisol, human placental lactogen, as well as urine estrogen & progesterone] are used to diagnose a spontaneous abortion. Several sonographic characteristics have been suggested as potential miscarriage predictors. These involve the presence & characteristics of the yolk sac⁽¹⁾, fetal heart rate, gestational sac size⁽²⁾, and decreased embryonic growth.

Our research was set out to determine how accurately cancer antigen 125 (CA-125) as well as beta HCG levels in the mother's blood may predict a miscarriage.

Threatened Abortion

In medical terms, an abortion is defined as the termination of a pregnancy before to the twentieth week of pregnancy. Abortions that don't end up being performed are classified as either missed or induced⁽³⁾.

Definition: If a urine or blood pregnancy test is positive & the cervical os is closed, there is also prohibited transfer of fetuses & no symptoms of fetal or embryonic mortality, then vaginal bleeding prior to twenty weeks of gestational age is regarded as a threatened abortion. According to the WHO, a threatening abortion occurs when a female experiences bloody vaginal discharge or

frank bleeding during the 1st half of her pregnancy with no experiencing cervical dilatation⁽⁴⁾.

Epidemiology

According to data published by the American College of Obstetricians & Gynecologists (ACOG), it is the type of miscarriage that occurs the most frequently. Nearly one in four pregnant women will experience some level of vaginal bleeding at some point throughout the first two trimesters of their pregnancies. Of the women who experience vaginal bleeding, around half will end up miscarrying their babies. Any pregnancy, regardless of the mother's age, lifestyle, comorbidities, or socioeconomic standing, is at risk of being threatened with abortion⁽⁵⁾. A woman's advanced age, a history of miscarriage, her father's advanced age, uncontrolled hyperglycemia, TORCH infections, uncontrolled thyroid disease, obesity, major stressors, the use of teratogenic medications & the chance of a subchorionic hemorrhage can all make it more likely that she will have an abortion⁽¹⁶⁾.

Symptoms and signs: It is possible for an individual to have pelvic pain, cramping in the abdomen, pelvic pressure, &/or back pain. In most cases, blood loss from the vaginal tract comes first, followed by cramping and abdominal pain several hours to days later. The greatest indicator of a future miscarriage is bleeding during pregnancy. Abortions will occur in more than fifty percent of cases where they are threatened. When a

person is diagnosed with a threatening abortion, the existence of fetal heart activity reduces the likelihood of the patient having a spontaneous abortion (7).

Etiology

It is not always possible to determine the precise reasons for a threatening or spontaneous abortion (6). Human embryonic development occurs in a low oxygen environment, in accordance with adult tissue criteria. Due to the numerous cell divisions that they undergo and the simultaneous exposure of their DNA, trophoblastic cells are particularly vulnerable to the damaging effects of oxidative stress. This event causes a rise in the synthesis of VEGF, which, in turn, can result in the inhibition of trophoblastic invasion. The observation of a low blood level of VEGF prior to embryonic death is suggestive of improper vascularization in the intervillary space & is connected with metabolic alterations at the level of the implantation zone. These modifications include a rise in the oxygen level, which is significant in light of the fact that the blood level of VEGF has been demonstrated to have an inverse correlation with the quantity of oxygen present (5).

Diagnosis

Beta-Human Chorionic Gonadotropin, along with additional biochemical markers, as well as a physical examination & medical history, are used to make the diagnosis (7).

Maternal age, previous pregnancies, medical history, and current disease should all be part of a comprehensive history taken from the mother. Assisting the patient in describing and quantifying the bleeding, as well as the onset and progression of symptoms and bleeding, moderating variables, and treatments tried up to the point of assessment, should all be included in the history of the present illness. In the general population, the probability of having a miscarriage is higher for women who are older. Having a miscarriage in the past is related to an increased risk of having another one in the future, particularly in older women (8).

A pelvic exam is required in order to ascertain the manner in which the abortion was performed, the total quantity of blood that was lost, the location of the bleeding, whether or not the cervix has dilated, & whether or not fetal tissue has been expelled. In the case of a possible abortion, the vaginal exam can show that the cervical os is closed as well as that no tissues have passed. There is typically no discomfort felt with cervical motion (9).

It is possible to find the pregnancy & ascertain whether or not the developing fetus is viable by using transvaginal ultrasonography. Also, the ultrasound can help rule out the chance of having an ectopic pregnancy then look for any products of conception that may have been retained. In a normal pregnancy, a yolk sac can be detected on an ultrasound approximately thirty-six days after the last menstrual period, and heartbeats can be seen approximately 65 days after the last menstrual period (10).

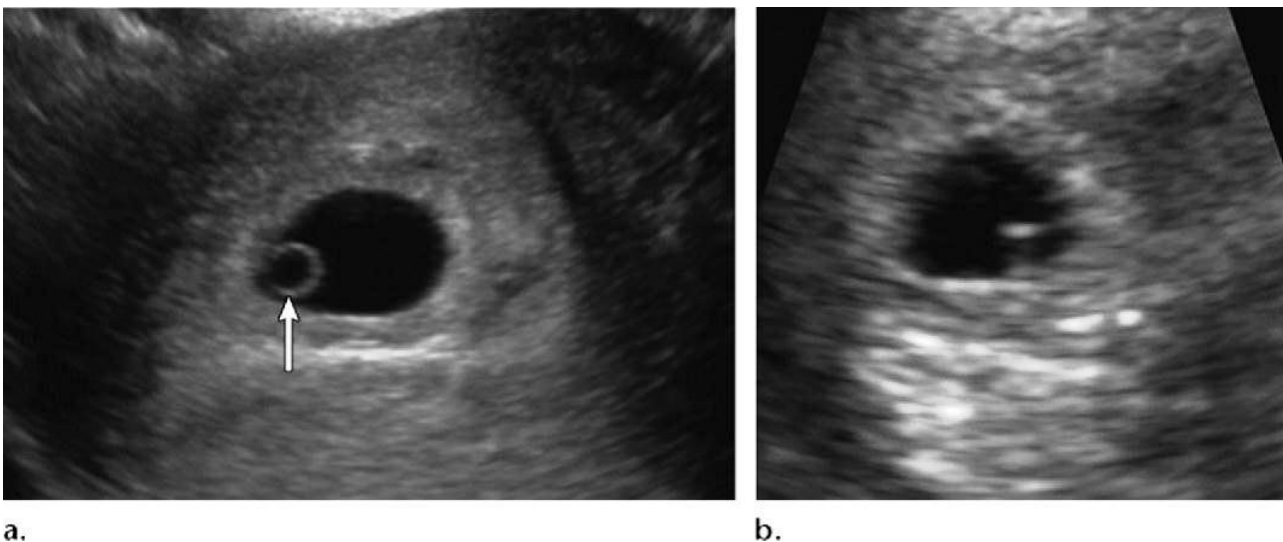


Figure (1): The endovaginal ultrasound scans taken during the early stages of pregnancy, all showed a healthy yolk sac (YS). (a) The echogenic ring that makes up the yolk sac can be seen as an eccentric circular structure within the gestational sac. The mean square deviation is twelve millimeters, which indicates that the gestational age is 5 weeks and six days. (b) The YS is shown as 2 parallel lines within a smaller gestational sac. These lines depict the leading edge as well as the rear wall of the sac. The mean square deviation is four millimeters, which indicates that the gestational age is five weeks and 0 days (11).

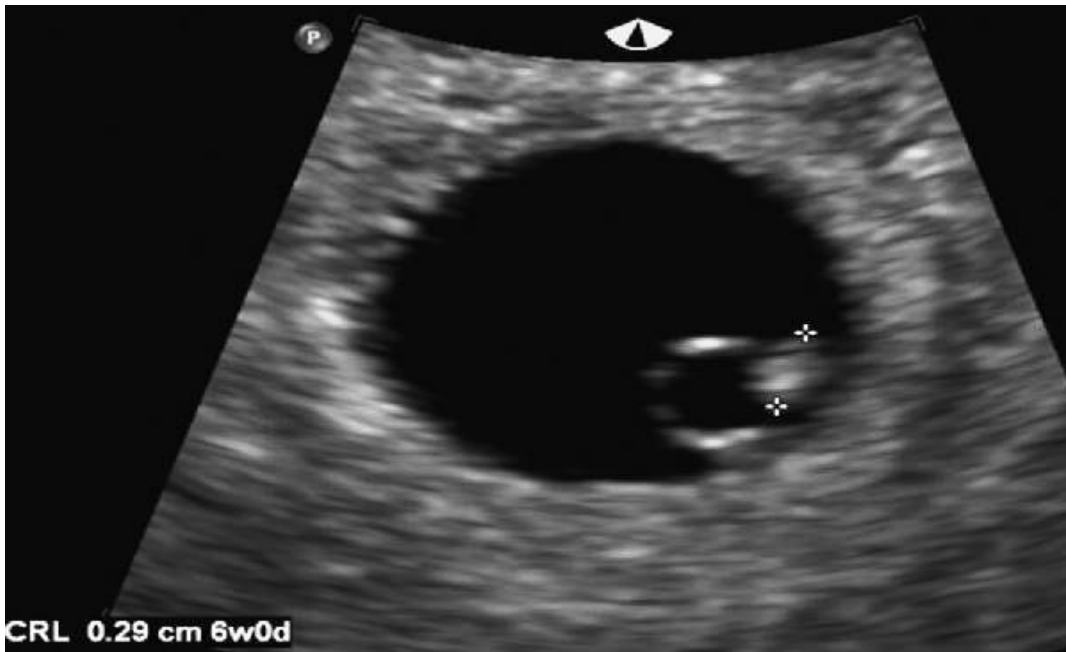


Figure (2): The CRL can be seen on an endovaginal ultrasound image taken of a pregnant woman. The CRL of the embryo measures 3 millimeters (between the marks on the caliper), and it is located near a healthy yolk sac. According to the CRL, the estimated number of weeks as well as days of gestation is six weeks and zero days ⁽¹²⁾. Additionally, the biochemistry of the maternal serum has been suggested as a potential predictor. In comparison with women who carried the pregnancy to term, those who had an abortion threatened during the first trimester but ended up having a miscarriage have lower levels of HCG in their serum. According to the findings of a prospective trial, a free beta human chorionic gonadotropin cut-off value of twenty nanogram per milliliter is sufficient to differentiate among healthy & unhealthy pregnancies ⁽¹³⁾.

Ultrasound detection of a gestational sac coincides with a beta human chorionic gonadotropin concentration of 1500 IU/milliliter to 2000 IU/mL. In 85% of IVF pregnancies, beta-human chorionic gonadotropin doubles within 48 hours. The concentration of beta-HCG in the blood begins to rise after ovulation, and by the time menstruation is about to begin, it has reached 200 IU/mL ⁽¹³⁾.

In the first trimester, there is little change in progesterone levels. Data from a variety of obstetric populations indicates that a blood progesterone concentration of 5.1 ng/ml is the bare minimum for a healthy first-trimester pregnancy ⁽¹⁴⁾.

Serum inhibin A along with HCG concentrations were significantly reduced in women who went through a miscarriage after a threatened abortion. Inhibin A at the cut-off of 0.553 multiples of the median was a predictor of abortion incidence. Finally, a sample of 128 women with symptoms but detectable fetal cardiac activity at presentation had significantly lower quantities of pregnancy-linked placental protein A (PAPP-A) than normal controls.

Nonetheless, pregnancy-linked placental protein A had only a 18.7% predictive value for miscarriage ⁽¹⁵⁾.

Table (1): Prognostic factors in cases of threatened abortion

Favourable prognostic factors	Adverse prognostic factors
History Advancing gestational age	Age of mother is older than thirty-four. A growing history of miscarriages
Sonography Fetal heart activity at presentation	Fetal bradycardia Empty gestational sac above fifteen to seventeen mm Discrepancy amongst gestational age in addition to crown to rump length
Maternal serum biochemistry Normal levels of these markers	Low beta human chorionic gonadotropin values CA125 over 43.1 IU/ml Beta Human Chorionic Gonadotropin increase under 66% in 48 hrs Free beta human chorionic gonadotropin value of 20 ng/ml Progesterone under 45 nmol/l in 1st trimester

Management

Doctors give bed rest & progesterone to women at risk of miscarriage. There's minimal evidence that progesterone helps women with impending miscarriages. However, one small study discovered that local progestogen treatment reduced uterine cramps faster than bed rest ⁽¹⁶⁾.

Transvaginal ultrasounds should be performed on a regular basis until either a viable intrauterine pregnancy is verified or the pregnancy progresses toward an eventual, incomplete, or complete termination. When ultrasonography has not confirmed an intrauterine pregnancy with heart activity, doctors may perform serial quantitative Beta Human Chorionic Gonadotropin testing to determine viability ⁽¹⁷⁾.

At least until the bleeding stops, several doctors recommend their patients rest their pelvic area and refrain from physically demanding activities. Clinicians should advise their pregnant patients to begin or maintain a regimen of prenatal vitamins containing folic acid ⁽¹⁸⁾.

Sonographic Evaluation of Threatened Abortion

An intrauterine or extrauterine pregnancy's viability can be determined with an ultrasound conducted in the early stages of pregnancy. Ultrasound (US) is the gold standard for detecting signs of fetal death ⁽¹⁹⁾.

Transvaginal ultrasonography (TVS) is commonly used to diagnose intrauterine pregnancy and monitor its progress because of its high-resolution pictures, low interobserver variability, as well as excellent reliability. Early pregnancy is examined by measuring the gestational sac, the yolk sac, the crown-rump length, the fetal heart rate, & the amniotic sac ⁽²⁰⁾.

Gestational Sac (GS)

Pregnancy progresses normally until an embryo with heart activity is found, and the size of the gestational sac is a reliable indicator of gestational age. There is a key discriminating sac size over which the fetal heart pulse should always be visible in a healthy pregnancy, but detection is typically possible as early as 10 mm. It has been suggested that a transvaginal sac size of 16–20 mm is more discriminatory than a transabdominal sac size of 20–30 mm for identifying ectopic pregnancies ⁽²¹⁾.

Yolk Sac (YS):

This telltale sign can help you identify an accurate gestational sac. When a gestational sac is larger than 8 mm in diameter, it is the first fetal structure often seen on TVS. It should be a healthy pregnancy.

The yolk sac is shown sonographically as a spherical structure with an anechoic center and a regular, well-defined echogenic rim. Up until about the tenth or eleventh week of pregnancy, the yolk sac expands in size from its typical 3 to 4 millimeters in diameter. When the amount of the yolk sac is outside the fifth to Ninety-fifth percentile, the risk of a spontaneous abortion increases dramatically. Yolk sac abnormalities & sizes greater than 9 mm may indicate severe fetal development issues ⁽²²⁾.

Embryology:

During a typical pregnancy, the three germ layers are set up during the third week of development during a process called gastrulation. The trilaminar embryo's ventral side can talk to the definitive YS. During week 4, as the embryo grows & folds laterally, the YS narrows at its base, giving rise to the yolk stalk (Figures 3 & 4) ⁽²³⁾.

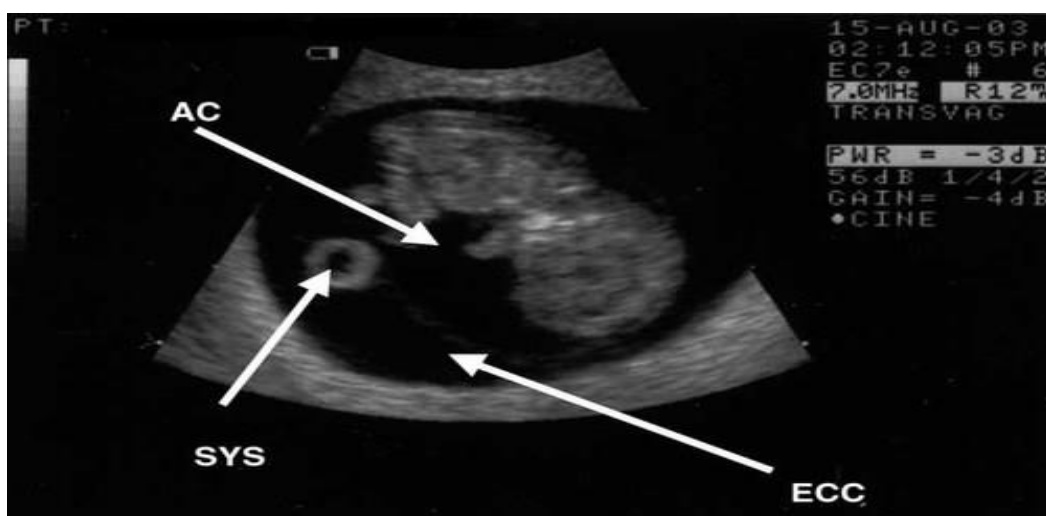


Figure (3): Transvaginal image of an 8-week pregnancy demonstrating the amniotic cavity (AC), exocoelomic cavity (ECC) & secondary yolk sac (SYS) ⁽²³⁾.

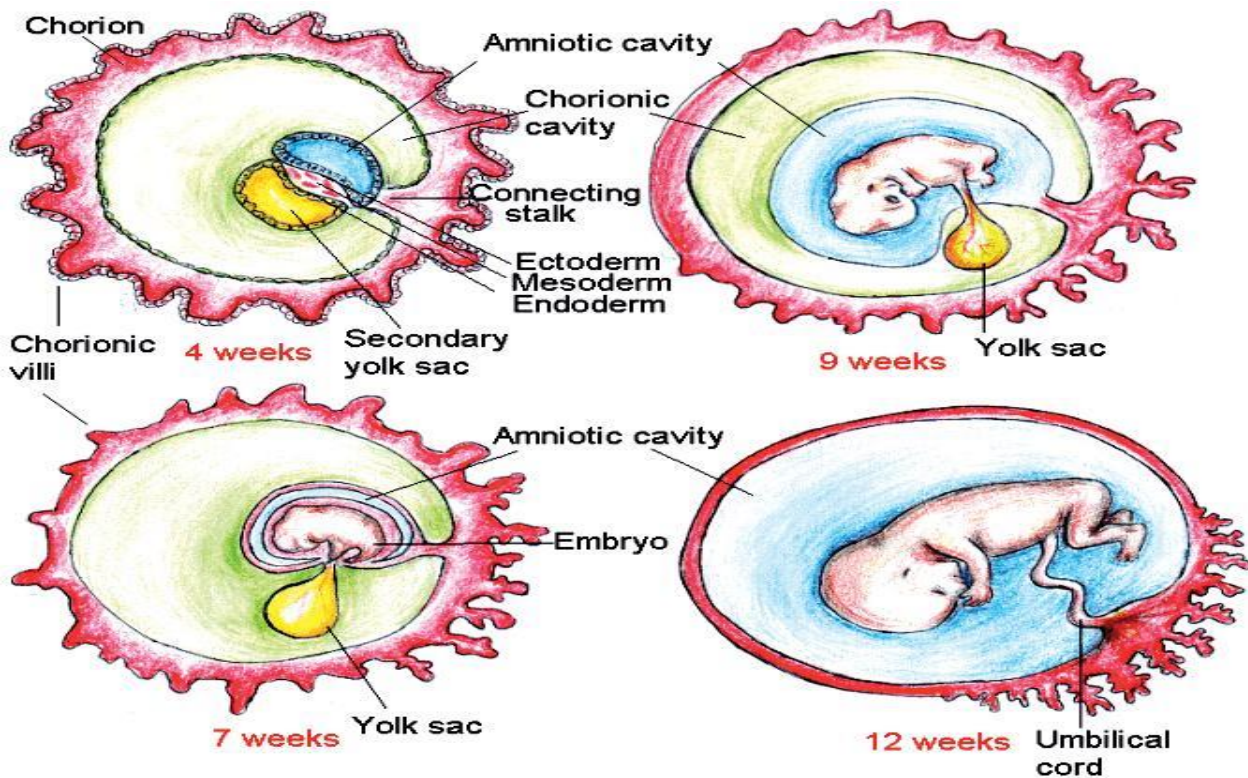


Figure (4): Diagram displays stages of the yolk sac & embryonic development ⁽¹³⁾.

After the ninth week of pregnancy, the yolk sac starts to change shape in a way that suggests that it is embryonic growth that causes it to involute, not mechanical compression by the growing amniotic cavity. This was found by using a transvaginal ultrasound to look at the yolk sac. This was done to see if there was a link between changes in the normal features of the YS diameter and pregnancy problems ⁽¹⁹⁾.

Echogenic and Calcified YS

On sonography, a calcified yolk sac may look like a ring that has an echogenic quality. It is not known to be linked to a live embryo that was created before the 12th week of pregnancy. In fact, a hardened yolk sac could only be seen in an embryo that had already passed away due to the fact that calcification of the yolk sac takes place just a few days after embryonic death has taken place ⁽²⁴⁾.

The distinction amongst a calcified yolk sac & an echogenic yolk sac is that echogenicity can be caused by a variety of elements in addition to calcium. An echogenic yolk sac is distinguished from an anechoic yolk sac. On the other hand, tissue harmonic imaging might prove to be a more reliable method for confirming these results. An echogenic yolk sac was only reported to be connected with embryonic death or malformations in one trial. It has also been noted in the relevant literature that the presence of an echogenic yolk sac does not foretell the death of an embryo or abnormalities in the embryo. In fact, it is predicted that prospective research on a massive scale will shed light on the diagnostic significance of echogenic yolk sacs ⁽¹⁸⁾.

Embryonic heart activity

It is common knowledge that the heart rate of an embryo will increase from an average of 97.7 beats per minute at thirty-six to thirty-eight days to 174.7 beats per minute at sixty to sixty-two days, & it is also common knowledge that the embryonic heart rate (HER) will continue to increase by approximately 4 beats per minute every day until eight weeks of gestation. This shows that the measurement of EHR by ultrasound may be helpful in timing early pregnancy in the first trimester, & in addition, bradycardia during the 1st trimester can be linked with a bad prognosis ⁽²⁵⁾.

Pregnancy Dating

The crown-rump length (CRL) is the only one of the parameters that has been shown to be reliable as well as reproducible in order to determine how far along a pregnancy is. Other parameters have been described. The CRL measurement enables an accurate diagnosis of the gestational age to be made when an EHR is available.

However, if EHR is not present and the CRL measurement is below seven mm, which corresponds to 7 weeks of gestation. It is absolutely necessary to determine whether or not the amniotic sac is present. Even if the CRL measures five or six whole weeks of gestation, the presence of an AS (amniotic sac) would date a pregnancy at least at seven weeks' gestation because the AS becomes evident on ultrasound at 7 weeks' gestation (Figure 5) ⁽²⁶⁾.



Figure (5): The double bleb indication, which consists of the amniotic sac & the yolk sac, with a glimmer of an embryo visible among them ⁽²⁶⁾.

Diagnosis of placental location:

The position of the placenta has a considerable bearing on the outcome of the pregnancy as well as on the risk of sickness and death for both the mother and the baby. Early detection of placenta previa enables clinicians to monitor the pregnancy more closely, which in turn lowers the risk of adverse pregnancy results, for instance, low birth weight babies, postpartum hemorrhage, gestational hypertension, & premature labor and delivery ⁽²⁰⁾. Due to the increased risk of morbid adherence to the anterior wall of the uterus as well as the posterior wall of the bladder, termination of pregnancy is recommended in cases of Cesarean section scar pregnancies, which account for 6.4% percent of ectopic pregnancies ⁽²⁷⁾ as well as are on the rise with the rising incidence of Cesarean deliveries ⁽²⁸⁾.

Cancer antigen 125 & beta human chorionic gonadotropin in the prediction of result in women with threatened miscarriage:

CA-125 (carcinoma antigen 125, cancer antigen 125, or carbohydrate antigen 125). The MUC16 gene was identified as the human genetic source of the protein mucin 16, commonly known as MUC16. Cancer antigen 125 stands out as an intriguing high-molecular-weight glycoprotein. Epithelial ovarian cancers that have serous or endometrioid differentiation are known to express this antigen. Serum levels of cancer antigen 125 above 35 IU/ml have been linked to a number of noncancerous gynecological conditions, for example, endometriosis, pelvic inflammatory disease, benign ovarian cysts, and ovarian hyperstimulation syndrome, suggesting that CA 125 is not a cancer-specific marker ⁽²⁹⁾.

CA 125 increases have potential as both a predictive & diagnostic indicator in symptomatic early pregnancy since they may reflect decidual, epithelial basement membrane, or amniotic membrane breakdown. Results from numerous studies assessing the prognostic usefulness of CA 125 in high-risk pregnancies have been largely dissatisfying ⁽⁹⁾.

Women with healthy pregnancies & women with threatened abortions were evaluated for CA 125 concentration & received follow-up care to learn about the result. Final CA 125 levels in patients who had an abortion were 58.17 ± 7.25 IU/ml, while those in healthy pregnant females who carried to term were 26.61 ± 1.76 IU/ml. Women who were threatened but did not terminate their pregnancies had a CA 125 level of 30.89 IU/ml. They came to the conclusion that blood CA 125 levels are a sensitive as well as specific predictor of loss of pregnancy in cases of threatened abortion because of their low cost, ease of availability, as well as lack of inconvenience ⁽³⁰⁾.

- **Consent for publication:** I attest that all authors agreed to submit the work.
- **Availability of data and material:** Available.
- **Competing interests:** None.
- **Funding:** No fund.
- **Conflicts of interest:** No conflicts of interest.

REFERENCES

1. **Berdahl D, Blaine J, Van-Voorhis B, Dokras A (2010):** Detection of enlarged yolk sac on early ultrasound is associated with adverse pregnancy outcomes. *Fertil Steril.*, 94 (4): 1535-1537.
2. **Bae S, Karnitis J (2011):** Triple ultrasound markers including fetal cardiac activity are related to miscarriage risk. *Fertil Steril.*, 96 (5): 1145-8.
3. **Abed A, Khudhur Y (2021):** The Value of Early Pregnancy Ultrasound Parameters in Prediction of First-Trimester Outcome. *Medico-Legal Update*, 21 (2): 1413-1418.
4. **Mouri M, Hall H, Rupp T (2023):** Threatened abortion. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing. <https://pubmed.ncbi.nlm.nih.gov/28613498/>
4. **Devall A, Coomarasamy A (2020):** Sporadic pregnancy loss and recurrent miscarriage. *Best Pract Res Clin Obstet Gynaecol.*, volume 69, Pp: 30-39. doi: 10.1016/j.bpobgyn.2020.09.002.
5. **du-Fossé N, van der Hoorn M, van-Lith J et al. (2020):** Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. *Hum Reprod Update*, 26 (5): 650-669.
6. **Devall A, Papadopoulou A, Podsek M et al. (2021):** Progestogens for preventing miscarriage: a network meta-analysis. *Cochrane Database Syst Rev.*, 4 (4): CD013792. doi: 10.1002/14651858. CD013792.
7. **Storey A, White K, Treder K et al. (2020):** First-Trimester Abortion Complications: Simulation Cases for OB/GYN Residents in Sepsis and Hemorrhage. *MedEdPORTAL*, 16: 10995. doi: 10.15766/mep_2374-8265.10995.
8. **Curry A, Williams T, Penny M (2019):** Pelvic Inflammatory Disease: Diagnosis, Management, and Prevention. *Am Fam Physician*, 100 (6): 357-364.
9. **Moradan S, Forouzeshfar M (2012):** Are abnormal yolk sac characteristics important factors in abortion rates? *Int J Fertil Steril.*, 6 (2): 127-30.

10. **Nyberg D, Filly R (2003):** Predicting pregnancy failure in 'empty' gestational sacs. *Ultrasound Obstet Gynecol.*, 21 (1): 9-12. doi: 10.1002/uog.33.
11. **Doubilet P, Benson C, Bourne T et al. (2014):** Diagnostic criteria for nonviable pregnancy early in the first trimester. *N Engl J Med.*, 369 (15): 1443-51.
12. **Pereira P, Cabar F, Gomez Ú, Francisco R (2019):** Pregnancy of unknown location. *Clinics (Sao Paulo)*, 74: e1111. doi: 10.6061/clinics/2019/e1111.
13. **Pereda J, Niimi G (2008):** Embryonic erythropoiesis in human yolk sac: two different compartments for two different processes. *Microsc Res Tech.*, 71 (12): 856-62.
14. **Puget C, Joueidi Y, Bauville E et al. (2018):** Serial hCG and progesterone levels to predict early pregnancy outcomes in pregnancies of uncertain viability: A prospective study. *Eur J Obstet Gynecol Reprod Biol.*, 220: 100-105. doi: 10.1016/j.ejogrb.2017.11.020.
15. **Bulletti C, Ziegler D, Maini M et al. (2001):** Effects of the vaginal progesterone gel crinone 8% on uterine cramps and bleeding associated with threatened abortion. *Fertility and Sterility - FERT STERIL.*, 76 (3): S184-S185.
16. **Grenache D (2020):** Current Practices When Reporting Quantitative Human Chorionic Gonadotropin Test Results. *J Appl Lab Med.*, 5 (5): 850-857. doi: 10.1093/jalm/jfaa082.
17. **Schindler A, Carp H, Druckmann R et al. (2015):** European Progestin Club Guidelines for prevention and treatment of threatened or recurrent (habitual) miscarriage with progestogens. *Gynecol Endocrinol.*, 31 (6): 447-9. doi: 10.3109/09513590.2015.1017459.
18. **Jauniaux E, Gulbis B (2000):** In vivo investigation of placental transfer early in human pregnancy. *Eur J Obstet Gynecol Reprod Biol.*, 92 (1): 45-9. doi: 10.1016/s0301-2115(00)00424-3.
19. **Gizzo S, Noventa M, Vitagliano A et al. (2015):** OC13.02: †Endometrial surveillance in tamoxifen users: accuracy of ultrasound versus hysteroscopic investigation—observational longitudinal cohort study. *Ultrasound Obstet Gynecol.*, 46: 28-28. <https://doi.org/10.1002/uog.15033>.
20. **Coulam C, Britten S, Soenksen D (1996):** Early (34-56 days from last menstrual period) ultrasonographic measurements in normal pregnancies. *Hum Reprod.*, 11 (8): 1771-4.
21. **Karataşlı V, Kanmaz A, İnan A et al. (2019):** Maternal and neonatal outcomes of adolescent pregnancy. *J Gynecol Obstet Hum Reprod.*, 48 (5): 347-350.
22. **Stampone C, Nicotra M, Muttinelli C, Cosmi E (1996):** Transvaginal sonography of the yolk sac in normal and abnormal pregnancy. *J Clin Ultrasound*, 24 (1): 3-9.
23. **Harris R, Vincent L, Askin F (1988):** Yolk sac calcification: a sonographic finding associated with intrauterine embryonic demise in the first trimester. *Radiology*, 166 (1 Pt 1): 109-10. doi: 10.1148/radiology.166.1.3275964.
24. **Tezuka N, Sato S, Kanasugi H, Hiroi M (1991):** Embryonic heart rates: development in early first trimester and clinical evaluation. *Gynecol Obstet Invest.*, 32 (4): 210-212.
25. **Detti L, Gordon J, Christiansen M et al. (2019):** Diagnosis of Placental Position by Early First-Trimester Ultrasound: A Pilot Study. *Reprod Sci.*, 26 (11): 1512-1518.
26. **Shahin A, Elmasry M, Gad M (2022):** Yolk sac size and shape, gestational sac diameter, and embryonic heart rate as prognostic factors of first-trimester pregnancy outcomes. *Menoufia Medical Journal*, 35 (2): 776-781.
2. **28.Timor-Tritsch I, Monteagudo A, Cali G et al. (2016):** Easy sonographic differential diagnosis between intrauterine pregnancy and cesarean delivery scar pregnancy in the early first trimester. *Am J Obstet Gynecol.*, 215 (2): 225.e1-7. doi: 10.1016/j.ajog.2016.02.028..
3. **29. Jacobs I, Fay T, Stabile I et al. (1988):** The distribution of CA 125 in the reproductive tract of pregnant and non-pregnant women. *Br J Obstet Gynaecol.*, 95 (11): 1190-1194.
4. **30. Odeh M, Tendler R, Kais M et al. (2010):** Gestational sac volume in missed abortion and anembryonic pregnancy compared to normal pregnancy. *J Clin Ultrasound*, 38 (7): 367-71.