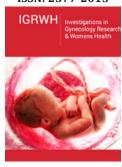


Pink Line of Pregnancy-Pleasure and Pains of Home-Based Pregnancy Tests

Suresh Kishanrao*

Public Health Consultant, India

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*Corresponding author: Suresh Kishanrao, Public Health Consultant, Bengaluru, India

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Abstract

Taking a home pregnancy test can be exciting, most of meaningful to take a decision of continuing or terminating the pregnancy, but it becomes sometimes stressful, especially when the lady is not sure whether she should trust the results. Knowing when and how to take a home pregnancy test and being knowledgeable of some of the possible drawbacks of home testing can minimize such stress. While many home pregnancy tests claim to be able to confirm pregnancy as early as the first day of a missed period and some even before that point. However, most home pregnancy test results are accurate if they are taken after the first day of a missed period. That's because shortly after a fertilized egg implantation in the uterine lining the placenta begins forming. The placenta makes the pregnancy hormone Human Chorionic Gonadotropin (HCG), which can be detected in blood and urine, present in the body only during pregnancy. The timing of ovulation makes a difference in the accuracy of a home pregnancy test. The ovulation can change from month to month even in the same woman. A fertilized egg also can be implanted in the uterus at different times. That can affect the timing of when HCG starts to be made and when it can be found with a home pregnancy test. Irregular menstrual cycles affect test results, as they make it hard to figure out when a period should start. Fertility medications or any other medicine that contains HCG and medicines containing Follicle-Stimulating Hormone (FSH), used to trigger the ovaries to release an egg also interfere with the results.

Material and methods: This article is based on 3 cases, monitored by the author. Two primary infertility cases showing positive home-based pregnancy test, after 18-24 months of hormonal therapy, D&C etc. One progressed to a full-time normal delivery, and another ended in gobbled fetus of the first gravida going through D&C and hormonal therapy. In the third case of post contraceptive secondary infertility, hormonal therapy has not helped, and the Ovulation cycle appears to have returned since just 2 months, but irregular periods have given the pains of false positive results in both periods.

Outcome: Only one case ended in happiness within 2 years observation and the other 2 cases of secondary infertility disappointed by repeated pregnancy test, are hoping to see the pink line proving pregnancy following home based pregnancy test, getting pregnant and finally having a baby in another year or so.

Keywords: Homebased pregnancy test kits-urine test; Control line; Colorless evaporation line; Blood test for HCG and ultrasound; False positive and false negative tests; Trusting the results; Corroborating with early pregnancy symptoms

Abbreviations: HCG/hCG: Human Chorionic Gonadotropin; FSH: Follicle-Stimulating Hormone; D&C: Dilatation and Curettage

Introduction

Usually, people take a home pregnancy test with a kit when they experience unexpected delays in the menstrual cycle. Although a delayed period is not always necessarily a case of conception, taking a test is a way to confirm it. Taking a home pregnancy test is becoming exciting, among literate women, in India but it is also perpetuating stress sometimes, especially when the lady is not sure to trust the results or in interpreting the pregnancy test. There are several reasons why a couple or the lady only decides to take a pregnancy test. Couple could be trying to get pregnant and hoping for a positive result. The couple might have experienced an issue in previous pregnancy like miscarriage, molar pregnancy, tubal pregnancy etc. Most importantly faced with their birth control methods. A couple may also propose to have a medical procedure or start a new medication that could be complicated by pregnancy. Most common among unwed pregnancies is to terminate the same as soon as possible. For a few

who don't want to expand their family early for various reasons like enjoying the bliss of newly married life or professional/career development demands. In India literate women are cultivating a habit of taking the test in a hurry as soon as they miss expected monthly periods, before consulting a Gynecologist to make decision to continue or terminate the pregnancy.

Pregnancy tests look for a special hormone called Human Chorionic Gonadotropin (HCG), that only develops in a woman's body during pregnancy. These tests can use either woman's pee or blood to look for HCG. At-home pregnancy tests that use your pee are the most common type. When used correctly, home pregnancy tests are 99% accurate. Many home pregnancy tests in India display results as lines in an indicator window. One line called control line shows that the test is working properly, and the other specifies whether the woman testing is pregnant or not. The presence of the line means she is pregnant, while the absence means she is not. Basically, any second line is considered a positive result, even if it's lighter than the control line. In fact, the control line is often darker than the test line. If a second line appears (pink/blue), even a faint one the test detects the pregnancy hormone in her urine. Sometimes young women testing for the first few times may confuse other lines on pregnancy tests for a positive result. The important thing to look for is a second line that develops color. A shadowy, shiny, colorless mark on a pregnancy test is not a positive. If anyone is ever unsure of the pregnancy test result, repeat using another test, and reconfirm.

Late marriages and delayed pregnancy interventions like use of Fertility medications or any other medicine that contains HCG might affect the test results too. The medicines like Novarel, Ovidrel, Pregnyl, and Profasi, used fertility treatments in females and in certain cases to raise testosterone levels in males and to help the testes descend in males who haven't yet reached puberty (Cryptorchidism). They are sued along with other fertility drugs to trigger the ovaries to release an egg. Drugs likw Bravelle, Fertinex, Follistim, and Gonal-F like medicines containing Follicle-Stimulating Hormone (FSH), taken by mouth, stimulate ovulation by causing the pituitary gland to release more Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), which stimulate the growth of an ovarian follicle containing an egg can also interfere the results. Certain malignancies of Pituitary gland, the liver, and the colon, also produce either HCG or HCG-related hormone and interfere with the result.

An ultrasound is used to confirm the milestones of your pregnancy and to check the fetal spine and other body parts for defects. An amniocentesis may be needed for an accurate diagnosis. Multiple marker screening is not diagnostic, it is never 100% accurate. Knowing when and how to take a home pregnancy test and being knowledgeable of some of the possible drawbacks of home testing can minimize such stress [1]. This article is based on 3 cases, monitored by the author, one ending in happiness and the other 2 still hoping to see the pink line following home based pregnancy test, getting pregnant and finally having a baby in FY 2024-25.

Case Report

Vani's pleasure after 3 years of wait

Vani a 28-year-old female married in 2021, conveyed the happy news of confirmation of her pregnancy in March 2024. An initial home-based pregnancy test, followed by scanning had confirmed the pregnancy this time and is due for partition in December 2024. But all this pleasant news did not come as easily as a young couple would expect. A year after marriage in April 2021 the year of Covid 19 Pandemic, the couple was trying to fulfill the desires of her mother and her in-laws. Taking Pregnancy test had become a routine for her every 3-4 months even if periods got delayed by about 2 weeks. The parental expectations and urgency had put enough pressure on the young lady married at the age of 25 years. The first suspected pregnancy ended in pain and displeasure by the end of 2022. Her Gynecologist put her on hormonal therapy after a D&C and made her to wait for 6 months. Another set of laboratory investigations & consultations led her to go into more depression and worry. At this time, around mid-2023 she consulted the author (more as Uncle than a doctor as women in India have reservations in consulting male doctors for reproductive issues!) and who advised her to stop all medications for next 4-6 months and share her details of her menstrual periods (the author was suspecting post contraceptive delay in normalization of ovulation), while her Gynecologist had advised some surgical intervention. Then, she naturally conceived what she termed as a" miracle" and was very happy. She attended a family function in June 2024 with 16 weeks of pregnancy. A Sac on 27 July provided further confirmation.

Pleasure of pink line & scanning confirmation followed by gobbling the foetus

Deepa & Dheeraj, another young couple married in 2019, postponed pregnancy intentionally until 2022. The young couple shared a pleasant news of pregnancy test turning pink (positive) in late June 2024 after 8 weeks of missing the due periods, followed by a scan report on 14 August confirming the pregnancy. The report read" Intrauterine gestation size 2.1x1.1cms, 4mm yolk sac, clear pouch of Douglas-clear- Diagnosing as "Single Pregnancy". But the pleasure not to continue for long! As there were no other natural pregnancy symptoms, a follow-up scan on 24 October 2023 diagnosed it to be "Blighted Ovum" and was advised a D&C, which was done very next week, after a second Gynecologist's consultation. Deepa was highly depressed and was put on Oral contraceptives for 4 months and is waiting anxiously for the happy news of pregnancy as of July 2024.

Pink line panic of secondary infertility

Bhargavi, another young lady of 29 years with a daughter of about 5 years, followed initially oral pills and then quarterly injectable contraceptive until end 2022. The couple have been trying to have a second baby since early 2023. The periods have not yet become regular suspecting post-hormonal secondary infertility. Giving an upper limit margin of 1 year for return of ovulation after injectable contraceptive, the wait continued. On 15 April 2024

Bhargavi shared the pregnancy test visual shown in the (Figure 1). Though she was elated as the kit directions aid even the faint pink

line of the test strip is positive. I advised her to for scanning that was done on 22 April 2024.



Figure 1: The faint pink line of the test strip is positive.

The result read "No evidence of intrauterine or extrauterine gestational sac. She was advised for a follow-up scanning after another fortnight and serial HCG correlation and repeat urine test. After a week she had heavy periods and the local gynecologist diagnosed it as endometriosis. Since then, her periods are irregular and heavy whenever happens. As she missed her pregnancy until 42 days, she tried another home pregnancy test which was negative. We were planning for a D&C and further investigations for secondary infertility but for her last periods on 12 July 2024 after 49 days, were like her normal periods before the first pregnancy. So, she has been advised to wait for another 3 months, as her ovulation cycles might return.

Discussion

From the very beginning of pregnancy, a woman's body starts to go through changes to support the cells that will develop into her baby. One thing that happens very quickly is the production of HCG. HCG levels start to build up once the fertilized egg implants in her uterus about 6 to 10 days after conception. There are two main types of pregnancy tests-urine tests and blood tests.

Urine test

Most literate women often, take a urine test at home with a home pregnancy test. This type of test is available over the counter and in a variety of price ranges.

Blood tests

To check for pregnancy are done in the laboratories and involve giving a sample of blood. A blood test for pregnancy may be needed for people who are having fertility treatments or have any other problem. These blood tests are slightly more sensitive than

urine tests because they can detect very small levels of HCG. That means they can provide a more accurate answer very early on in pregnancy within seven to 10 days after conception. For this test, your blood sample is taken and sent to a lab for analysis. Results might take anywhere from a few hours to two days. A blood test confirms pregnancy first because it can detect a smaller amount of HCG as compared to a test that uses pee.

Ultrasound

The other way to confirm a pregnancy is by using an ultrasound There are several reasons why a woman might take a pregnancy test. She could be trying to get pregnant and hoping for a positive result. She might have experienced an issue with her birth control methods especially oral pills or quarterly hormonal injections. A lady might even be about to have a Medical Procedure (MTP) or start a new medication that could be complicated by pregnancy [1]. Different line colors don't signal a pregnancy complication or a faulty test, assuming you followed the instructions properly. Rather, it usually means that levels of the pregnancy hormone Human Chorionic Gonadotropin (hCG) are high enough to trigger a positive result, but not to darken the test line very much. HCG levels take time to build in our body following conception and implantation [2], (Figure 2). It is always better, and many health experts recommend testing the first sample in the morning to avoid diluted urine. Sometimes the pregnancy test's second line is so faint, you can barely see it. Still, if you detect the line within the recommended timeframe (1 & 10 minutes), one must consider it a positive result. At-home pregnancy tests check pregnant woman's urine hCG, which ramps up quickly in the woman's body after fertilization. The lines on a pregnancy test develop color if hCG is detected.

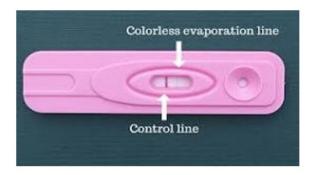


Figure 2: The lines on a pregnancy test develop color if hCG is detected.



Figure 3: If hCG is present in the urine, both the test line and control line will appear pink. Two pink lines indicate a positive result, even if one line is fainter than the other.

Special reagents are deposited at 2 locations on a pregnancy test strip, forming the "control line" and the "test line." These reagents appear as off-white/slightly gray lines on the test stripbefore or after use. In a First Response™ pregnancy test, these reagents are gold nanoparticles, which appear pink in color. They are responsible for turning the lines pink once the test is used. If no hCG is detected in a woman's urine, only the control line will appear pink colored. A pregnancy test will be positive even if the lady has an ectopic pregnancy (Figure 3).

Some test kits are very sensitive and detect lower levels of hCG as early as 6 days sooner

Research in Africa found that the false-positive tests resulted from performing the UPreg tests in test tubes that sufficient residual from the pregnant women's high ß-HCG levels had remained in the test tubes to cause subsequent false-positive results. The same may still happen in the laboratories in developing or resource constraint countries due to lack of appropriate cleaning and quality-control procedures. Of-course pregnancy can now be reliably diagnosed with inexpensive, disposable and simple home test kits. These tests must not only be used properly, but with quality control measures if used in laboratories [3].

A false-positive/false negative pregnancy test

Many home pregnancy tests boast of 99% accuracy, but false positive & negative tests are reported though rare and lead to pain, disappointment, or aspirations respectively. The users must be aware of the limitations of the test.

Common Errors for Getting False Positive Results Using Pregnancy Test Kits

User error

Many home pregnancy tests boast a 99% accuracy rating. This figure is usually displayed in a large, eye-catching font on the front of the package. However, that the rating is based on Trusted Source tests performed by lab technicians which is explained in the fine print that folks most of us do not notice or read meaning the producer expect a "perfect use" level of accuracy in a "typical use" environment prone to human error. Some of the most common mistakes are using an expired test, taking the test too soon after sex, taking the test too soon after a recent abortion, miscarriage, or childbirth, drinking water or other fluids before taking the test, removing the dipstick from the urine outside and checking the test results outside of the recommended time frame.

Recent pregnancy

The human body produces the hormone hCG throughout pregnancy, peaking toward the end of the first trimester and gradually declining until pregnancy ends. Afterward, the hormone can remain in your blood and urine for up to 60 days, based on whether the pregnancy was carried to term. The higher your hCG levels are, the more time it will take to return to your prepregnancy baseline. Retaining the Products of Conception (RPOC) and removing the lingering tissue or blood clots may be required. Symptoms of RPOC include heavy, irregular, or persistent vaginal

bleeding, unusual vaginal discharge, lower abdominal or pelvic pain, and fever. A D&C may have to be done as treatment.

Chemical pregnancy

"Chemical pregnancy" isn't a medical term but occurs when elevated hCG levels are the only indication of implantation. The fertilized egg may develop into a blastocyst about 6 days after implantation. However, it doesn't develop into an embryo, which occurs about 12 days after implantation. No treatment is necessary. Chemical pregnancies are not uncommon. Old data in a 2017 study suggests that this accounts for about 8% to 33% of miscarriages.

Ectopic pregnancy

Ectopic pregnancy occurs when a fertilized egg implants grows outside of the main cavity of the uterus, usually in a fallopian tube, though it can also occur in the cervix, ovary, or abdominal cavity. Pregnancy isn't viable because there's no place for it to develop outside of the uterus. Its symptoms include i) unusual vaginal bleeding, ii) low back pain iii) mild abdominal or pelvic pain or mild cramping on one side of the pelvis and pressure on the rectum. If it ruptures may exhibit sharp, sudden abdominal or pelvic and shoulder pain weakness, dizziness, or fainting.

Molar pregnancy

Molar pregnancy results when the Gametes which are haploids (contain single set of chromosomes of Ovum and Sperm) do not join correctly during fertilization to create a zygote, a diploid cell containing two complete sets of different chromosomes. There are two types of molar pregnancies. A complete molar pregnancy occurs when sperm fertilizes an egg with incomplete or malfunctioning chromosomes resulting in fluid-filled cysts inside the uterus, and an embryo doesn't begin to form. A partial molar pregnancy occurs when two sperm fertilize an egg, resulting in a zygote with three complete sets of chromosomes resulting in an abnormal tissue to form inside the uterus, resulting in an embryo formation, that doesn't grow or develop. In either case, it needs to be removed. Molar pregnancy is considered rare but varies in different countries. In India and the Middle East, the incidence of molar pregnancy is estimated at 1 in 160 pregnancies, as compared to one in 600 in UK, and one out of every 1,000 to 1,200 pregnancies in the United States [4].

Certain medications

Fertility medications like Novarel, Ovidrel, Pregnyl, Profasi use hCG to help stimulate ovulation, and for weight loss which might give false positive test results.

Certain underlying conditions

Conditions like anovarian cyst, Tubo-ovarian abscess, adenomyosis, choriocarcinoma, Persistent Trophoblastic Disease (PTD), Placenta Site Trophoblastic Tumor (PSTT), quiescent Gestational Trophoblastic Disease (GTD) and even some perimenopausal, menopausal, or postmenopausal women experience elevated hCG levels and may give false positive pregnancy tests.

Consequences of False Positive Pregnancy Tests

Association between acute pyelonephritis and false positive test

In 2022 A 25-year-old woman using injectable fertility medicines presented with amenorrhea of 7 weeks and homebased pregnancy test positive and acute urinary infection symptoms. For immediate relief I and put her urine alkalizing medicines, antibiotics-Cefazoline and cholinergic drugs containing acetaminophen and hydrocodone and asked to get done a scan of Pelvis and abdomen. On follow-up, after 3 days she had a negative serum pregnancy test. Abdominal Computed Tomography (CT) scan had identified a left-sided staghorn calculus resulting in partial ureteric obstruction and hydronephrosis. This case of a false-positive urine pregnancy test had an association with the delay in the diagnosis & treatment of acute pyelonephritis.

Evaporation line after recommended time of reading the test

Our case of Bhargavi shared here is an example of the pregnancy test reading after the recommended time had elapsed. It results in what looks like a positive test result, but in most cases, this is not a positive pregnancy test, but an evaporation line left by the urine after the recommended time for reading the test.

Post miscarriage/abortion false positive result

In December 2021 a young couple married for 3 years approached me with great happiness and a positive pregnancy test at home for confirmation. I knew, the lady had a miscarriage of 6 weeks pregnancy in end October 2023, I asked for scanning, which showed no signs of pregnancy (expected: A curved embryo with a tail, looking a bit like a small tadpole and the developing limb buds). This proved to be a case of a false home urine pregnancy test positive very soon after a miscarriage. The couple was disappointed but after an abstinence of 3 months, a true positive test and pregnancy was confirmed.

The scientific explanation for this incidence is once a fertilized egg implants into the uterine wall, the body secretes hCG, and starts to decline as soon as 5 days after a miscarriage or medical abortion. However, in some cases it may remain at a readable level for longer. HCG will generally start to fall and return to their baseline or prepregnancy levels, in up to 6 weeks, depending upon the duration of pregnancy, longer it is woman will have more in her bloodstream [5]. In an incomplete miscarriage (some tissue from the pregnancy in the uterus) may allow continuation of producing hCG resulting in positive test. Such individuals may have to undergo a D&C to remove any remaining pregnancy tissue.

Common Errors for Getting False Negative Results using Pregnancy Test Kits

Despite technological advances, there's still a lot of mystery about the menstrual cycle. There can be as much as a 13-day difference in when ovulation occurs, and a woman may think she is 4 weeks pregnant, when she is only 2 weeks along. The common errors leading to a false Negative Pregnancy Tests are:

Low hormone levels

Sometimes, levels of the pregnancy hormone (hCG) early in pregnancy aren't high enough especially If a woman conceives later in her cycle, her hormone levels may not be high enough at the time of her missed period. Pregnancy bleeding, recent hormonal contraceptive use, or breastfeeding can all interfere with accurately knowing your dates as well. A retest after another 10-12 days may resolve the issue. Continued missing of periods, may call for consultation to rule out any complications.

Ectopic pregnancy

Sometimes (2-3%) an ectopic pregnancy can show up as negative on a pregnancy test. In the detection of ectopic pregnancies, qualitative assays with a threshold β hCG of 10IU/L would have a sensitivity of 99.5% and tests with a threshold β hCG of 25 to 50IU/L would detect 91% to 96% meaning without that 1-10% results may be false positive. Seeking medical consultation is advisable if the pregnancy test is negative despite having having i) severe pain low in your abdomen or on one side ii) dizziness or lightheadedness iii) bleeding or spotting iv) nausea and vomiting [6].

Lifestyle factors

Many non-reproductive factors can wreak havoc on a woman's menstrual cycle. Issues like Stress, Malnutrition, too much caffeine, breast feeding and not eating enough food can delay a woman's periods as is the case in our case of Bhargavi. Sudden lifestyle changes, like intense exercise or working the overnight shift on the job, affect too

Medical conditions

Medical conditions such as Polycystic Ovary Syndrome (PCOS) or thyroid problems may cause irregular cycles and missed periods, very light periods, very heavy periods, and skipped periods altogether.

Medications

Birth control pills and injection also cause irregularities in cycle. Other drugs used in hypertension, or allergy can throw off her cycle.

Consequences of Fals Negative Pregnancy Test 1

Hydatidiform molar pregnancy

My memory goes back to 1969 during my internship, when a 26-year-old rural woman primi-gravida 1, presented to the casualty OPD around 2200hrs., married for about 18 months reporting 20 weeks of amenorrhea, lower abdominal pain, vomiting, and 4 weeks of irregular vaginal bleeding. Home based pregnancy test done around 8th week had shown positive result. Physical examination revealed that she was anxious and in moderate discomfort, and her heart rate and blood pressure were elevated. Her abdomen was soft, with a tender lower abdominal mass. The pelvic examination by a staff nurse revealed a cervical OS that was open 2cm, without mild active bleeding, and a pelvic mass the size of a 12-week-old fetus. Repeat pregnancy tests of urine and serum for the beta

subunit of human chorionic gonadotropin (β -hCG) were negative. Ultrasonography revealed a heterogeneous, intrauterine mass with cystic components, a finding consistent with molar pregnancy. Prof & HOD of Gynae department explained to the husband and the parents of the girl about the case and advised for an immediate dilation and curettage which she did undergo. Pathological examination revealed a complete hydatidiform mole.

A falsely low β -hCG result in gestational trophoblastic disease results in delaying the diagnosis and the response to therapy, recurrence, or the persistence of disease. Dilution of the serum or urine sample in the appropriate setting can prevent delays and complications from a falsely low or negative pregnancy test.

A Case of a negative urine pregnancy test in a multiple gestation pregnancy

This experience goes back to my postings in a rural health center in 1975. A 34-year-old rural female with (Gr 3, Para 2 FTNDS), with no chronic medical conditions like hypertension or diabetes presented to our OPD reporting several days of abdominal discomfort, nausea and vomiting. She was actively trying to conceive for want of a male child without the use of any contraceptive despite the youngest daughter being just 18 months old. Her Last Menstrual Period (LMP) was approximately eight weeks prior and homebased pregnancy has turned negative. She had 2 prior uncomplicated pregnancies and live births in the same center. The patient was provided a prescription for antiemetics and instructed to get an ultrasound in the district headquarters. At a follow-up visit after 4 weeks, the abdominal discomfort was reduced, pelvic cramping, or vaginal bleeding was not there. Another pregnancy test had shown negative result. As she had not gone for ultrasound, she was asked to get it done immediately, which she did the same day. The transabdominal report revealed at least two gestational sacs and two fetal poles, inferred as a twin gestation. Fetal heart rates were reassuring, and measurements revealed EGA between nine and ten weeks for both [7].

The literature review also found mention of the urine pregnancy kit tests failing to detect pregnancy, due to "The hook-effect" a rare but important phenomenon, rendering the kit tests false negative due to an improper antigen-antibody ratio [8].

Tips To Follow When to Take the Test Result

In general, the best time to take a test is with the first morning pee, though, some pregnancy tests are sensitive enough to detect HCG no matter what time of day the test is taken. When possible, try to wait until it's been three hours since your last pee before you take the test if take two pregnancy tests to confirm and get the same result [9].

Most women, get a positive result from an at-home test as early as 10 days after conception. For a more accurate result, it is better to wait until after lady missed her period to take a test. If a test is taken too soon, it could be negative even if she is pregnant. If you get a negative test and then miss your period, take another test.

- a. Wait a couple days and test again if you still haven't gotten your period.
- b. Take the test first thing in the morning or after holding your urine for several hours.
- c. Open the test envelope and use it right away after checking its expiration date [10].

Levels of hCG rise quickly doubling every few days in the first weeks of pregnancy. Only pregnant woman has a placenta, that produces hCG. which develops shortly after a fertilized egg attaches to her uterine wall. Levels of hCG generally double every 24 hours during the first 8 weeks, peak around 10 weeks, and then decrease. Considering that pregnancy tests detect hCG in urine, the test line gets darker as the first trimester progresses (Figure 4), [11].



Figure 4: The test line gets darker as the first trimester progresses.

Conclusion

Despite a great advancement in pregnancy tests and their producers calins of 99% accuracy, every woman must corroborate the test results with early symptom & signs of pregnancy like-i) Sore breasts ii) Implantation bleeding iii) Slight cramping iv) Frequent urination v) Mood swings vi) Headaches vii) Nausea viii) Food cravings or aversions. Sometimes these symptoms may be psychological, especially among secondary infertility cases needing consultations with a Gynecologist.

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