

CHAPTER NINE

Serology of Human Chorionic Gonadotrophin Hormone (HCG) (Urine pregnancy tests,

Learning Objectives

➤ **At the end of this chapter Students will able to:-**

- 0 **Explains the factors that affect urine pregnancy test,**
- 0 **Know urine specimen collection**
- 0 **Differentiate method of determining HCG**

Outline

- **Introduction to HCG**
- **Factors that affect urine pregnancy test,**
- **Urine specimen collection**
- **method of determining HCG**

Introduction

- Pregnancy is the period during which a woman carries a baby with in her body before giving birth.
- Pregnancy begins with conception-that is, the fertilization of an egg by a sperm.
- The fertilized egg is called **zygote**.
- Progesterone maintains the uterus during the pregnancy and prevents any further release of eggs from the ovary

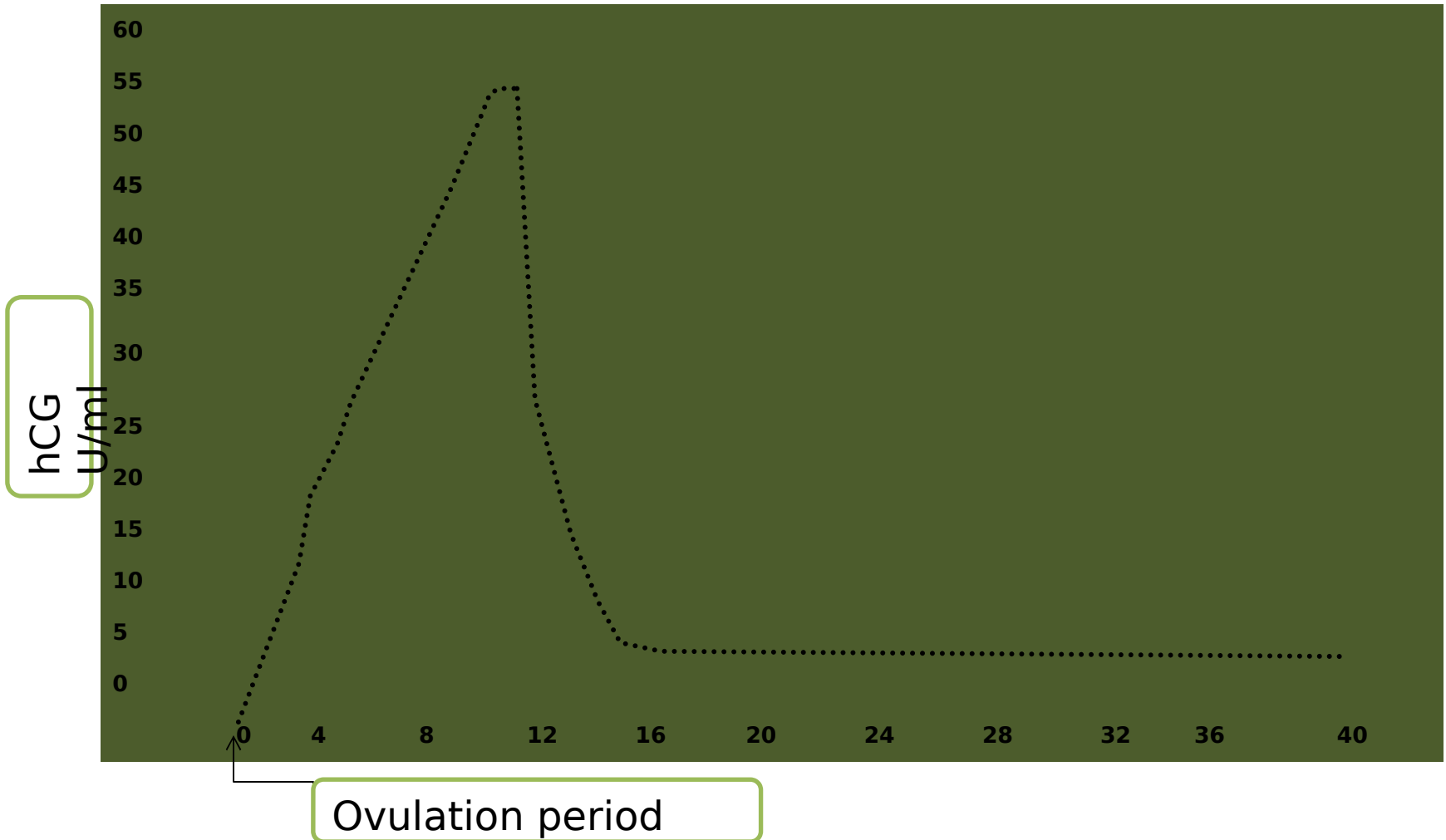
Introduction...

- During pregnancy, the chorionic membrane of the placenta produces a hormone **called human chorionic Gonadotrophin (hCG)**, which stimulates the secretion of progesterone, by the ovary.
- Human chorionic Gonadotrophin (hCG) is synthesized in large amounts by the **placenta**, and it appears in **urine, blood, amniotic fluid and serum** relatively soon after implantation of the developing embryo.
- The presence of this hormone serves as basis for pregnancy testing.

Introduction...

- Production of hCG **increases steadily during the first trimester**, peaking around the 10th week of gestation.
- Levels then **fall to less than 10%** of the first trimester levels during the remainder of the pregnancy.

Introduction...



Introduction...

- The urine and serum of pregnant women contain **high concentrations of human chorionic gonadotrophin (hCG)** produced by trophoblast, which provide the basis of tests for the diagnosis of pregnancy.
- Specific and sensitive analytical methods for the β -chain sub unit of hCG permit the detection of pregnancy as early as 8 days after ovulation (1 day after implantation).
- hCG concentrations climb early in pregnancy, reaching a maximum by 8 to 10 weeks of gestation.

Application of pregnancy tests

- **Pregnancy tests are important in investigation of suspected:**
 - ✓ **To detect early pregnancy.**
 - ✓ **To determine the adequacy of hormone production in high risk pregnancies (for example, habitual abortion)**

Application of pregnancy tests...

- **Ectopic disease:** the implantation of the fertilized egg in the **extra uterine space**. Example in the **fallopian tube**
- **In threatened abortion:** to conform the abortion is complete or not. In this case the quantity of hGC is decreases gradually.
- **In the infection of hydated mole:** to give treatments in the way not harm the fetus to check before mechanical treatment. Example X-ray.

Detection of hCG in urine

- A number of serologic tests have been used in pregnancy testing; each designed to **detect minute amounts of hCG** when it appears in the urine during the first few weeks of pregnancy.
- The methods most commonly used now are based on the **agglutination inhibition test**
 - ✓ Developed by Noto and Miale (1964)

Detection of hCG in urine...

- The amount of hormone excreted in the **urine** is almost the same as that found in the **blood**.
- By about the **8th day** after the first missed period during the pregnancy, the hCG level is about 1000IU/L (1IU/ml), and by the **12th day** it is about 2500IU/L(2.5 IU/ml).
- Laboratory pregnancy tests are based on detection of rapidly rising levels of **hCG in urine**.

Specimens

Urine specimen:

- An early morning specimen is preferable because this is the **most concentrated** and will therefore contain the **highest level of hCG**
- The urine must be collected in
 - ✓ a clean container, which is free from the all traces of detergent.
- If the specimen cannot be tested immediately it should be refrigerated at 4°C, but for not longer than 48 hr's.
- Specimens preserved with **boric acid** are also suitable for testing.

Urine specimen...

- If the urine is cloudy it should be flittered or centrifuged and the supernatant fluid used.
- Specimens that are heavily contaminated, or contain large amounts of **protein or blood**, are not usually suitable for testing.
- There are different types of commercially produced kits for pregnancy tests and each of this is with the technique and precaution with instructions.

Types of urine testing kits

1. Rapid latex slide test of the inhibition
 - I. Direct agglutination test
 - II. Indirect or inhibition test
2. Heamagglutination technique (tube tests of the inhibition type)

Rapid latex slide test of the inhibition

I. Direct slide agglutination test (Direct latex slide tests).

- Recently introduced techniques,
- More sensitive than the inhibition tests

I. Direct slide agglutination test

Principle

- *In the direct slide test, the latex reagent consists of particles coated with anti hCG antibodies.*
- *This reagent is mixed directly with the urine there will be agglutination, which is visible to the naked eye if the urine contains the hormone hCG.*
- *If not there is no visible agglutination.*

I. Direct slide agglutination test...

Procedure

1. Prepare clean, dry, detergent free slides.
2. Take one drop of early morning urine in Pasteur pipettes and drop on slide.
3. Take 1 drop of reagent (anti-hCG antibody).
4. NB. Don't use an expired reagent.
5. Mix gently; and see for agglutination

I. Direct slide agglutination test...

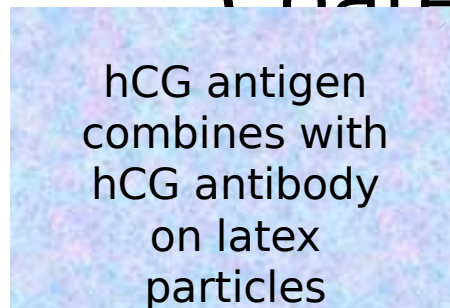
- Now a day people use random urine for pregnancy test. Because they consider as the hCG hormone may present at any time in the urine. But the morning urine is more preferable due to content of high concentrated hCG.

- If hCG is present in the urine, it will combine with the antibodies and causes **agglutination of the latex particles.**

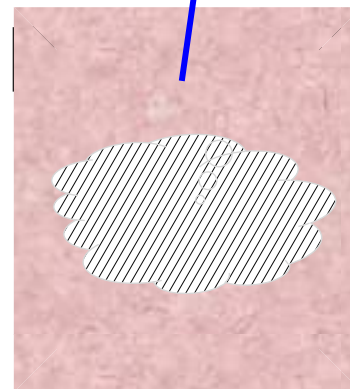
Urine latex reagent Result

(hCG **antibody**)

Agglutination



Coated ()



I. Direct slide agglutination test...

- In the direct slide test, therefore, agglutination of the particles indicates **positive** tests and **no agglutination** indicates a **negative** test.

II. Indirect latex slide test **(inhibition latex slide test)**

Principle:

- ***Colored latex or other visible particles coated with hCG, antibodies to hCG and urine are mixed with particles. Negative urine results in visible agglutination; and positive urine results, presence of hCG in urine inhibits agglutination (or protein flocculation).***

II. Indirect latex slide test...

Reagents:

- Antiserum containing anti-hCG-antibody
- Latex reagent containing polystyrene particles coated (sensitized) with the hCG antigen.

II. Indirect latex slide test...

Procedure:

1. Take clean slides which is free from detergent
2. Mix a drop of urine with one drop of antiserum on slide.
3. Add latex reagent
4. See for positive or negative test.

II. Indirect latex slide test...

- If hCG is present in the urine it will combine with the anti-hCG antibody. This will leave no antibody free to combine with the latex hCG and therefore there will be no agglutination of the latex particles.
- If hCG is present in the urine it will combine with the anti-hCG antibody. This will leave no antibody free to combine with the latex hCG and therefore there will be **no agglutination** of the latex particles.



Urine	+	antiserum hCG	+	latex reagent hCG
		Result		
		antibody		Ag coated particles
		No		

II. Indirect latex slide test...

- If there is no hCG in the urine, the antibody will be free to combine with the latex hCG and cause agglutination of the latex particles.

Urine	+	antiserum hCG	+	latex reagent hCG	→	Result
		Ab		Ag coated particles		
No Agg						

Inhibition tube agglutination test

Principle

- **Colored visible particles (RBCs) coated with hCG, antibodies to hCG, and urine is mixed with particles. Negative urine results in visible agglutination; and positive urine results, (presence of hCG in the urine) inhibits agglutination (or protein flocculation).**

Inhibition tube agglutination test...

- This technique uses RBCs coated with the hCG molecules.
- These particles are mixed with urine sample and then with a solution containing antibodies to hCG sub units.
- In the absence of urine any hCG, the antibody reacts with the hCG coated particles and causes agglutinations.
- When hCG is present in the urine sample, it will react with and neutralize the antibody, thus inhibiting particle agglutination.

Reagent

12/18/2021 • **Anti-hCG antiserum.** By: Olayinka Husen

Inhibition tube agglutination test...

Procedure

- **Take clean non-broken and detergent free tube.**
- **Add urine into the tube.**
- **Add anti-hCG antiserum.**
- **Mix well. If there is hCG there will be reaction.**
- **Add RBC coated with hCG.**
- **Mix the contents. Leave at room temperature (20 to 28°C) for 1 - 2 hours to allow the red blood cells to settle to the bottom of the tube.**

Inhibition tube agglutination test...

- **If hCG is present in the urine it will react with the antiserum leaving no antibody to agglutinate the RBC coated with hCG.**
- **If the urine contains no hCG the anti-hCG, antibody will react with the hCG on the red cells and causes their agglutination (Heamagglutination)**
- **The agglutinates will settle and be seen covering evenly the bottom of the tube.**

Sensitivity and Specificity of PT test

- **Sensitivity:** a term used to describe the probability that a laboratory test is positive (that is greater than the upper limit of normal) in the presence of disease.
- Sensitivity is defined as true positives divided by the sum of true positive and false negative

Sensitivity and Specificity of PT test...

- **Specificity** is used to describe the probability that a laboratory test will be negative (that is, within the normal range) in the absence of disease.
- Specificity is defined as true negative divided by the sum of true negative and false positives

Factors that affect pregnancy tests

- ✓ **The time in the pregnancy when the test is carried out.**
- ✓ **The presence of excessive amounts of protein or blood in the urine may cause false positive results.**
- ✓ **Detergent contaminated urine**
- ✓ **Turbidity of the specimens**
- ✓ **Bacterial contamination of the urine may cause unreliable results.**
- ✓ **Drugs, which may cause false-positive results, include**

Any question so far!!!

