EVALUATION OF IMMUNOLOGICAL PREGNANCY TEST

by

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Recent advances in immunoreproduction have solved the problem of early detection of pregnancy.

During pregnancy Langhans cells of the chorionic layer of the placenta produce chorionic gonadotrophic hormone. The antigenic nature of this hormone was first demonstrated by Collip and others (Collip, 1934 and Salye *et al* 1934). Since then various workers (Henry *et al* 1964, Hutcherson *et al* 1964, Jacobson *et al* 1965) have used an immunological pregnancy test for the detection of high concentration of hormone in blood and urine. The biological tests (Aschheim *et al* 1928 and Zondek *et al* 1945) which have been commonly used so far, have limitations as they require a well bred animal colony and longer time for detection.

The present study was carried out to evaluate the tube as well as the slide immunological pregnancy test. This test is based on the principle of inhibition of agglutination of human chorionic gonadotrophin (H.C.G.) coated latex particles by anti H.C.G. serum which has been fixed by the test urine.

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Material and Methods

One hundred and eighty-four morning specimens or 12 hours urine samples were collected from cases suspected of pregnancy.

The tube test was done in 34 samples as limited supply of sera were available.

The slide agglutination test was carried out in 150 samples. Out of these, in 100 samples the pH and specific gravity tests were also done. Confirmation of slide tests was done by clinical criteria and by rat ovarian hyperaemia test in 101 samples.

Technique

*Tube Test**

1. Shake and centrifuge the specimen at 1,000 g for 3 minutes.
2. Add 0.5 ml. of H.C.G. antiserum to 0.5 ml. supernatent.
3. Incubate the mixture at 37°C for one hour.
4. Add 1.0 ml. of latex antigen and incubate at 37°C for 2 hours.
5. Centrifuge the mixture at 1,000 g for 2 minutes.

*Ortho immunological pregnancy tube test.

6. Compare supernatent portion of the test system with the turbidity standard.

7. Turbidity equal or greater than standard: Postitive.

8. Turbidity less than standard: Negative.

*Slide Test***

1. Place a drop of urine on a clean black slide.

2. Add one drop of antiserum, mix and rotate for 30 seconds.

3. Add 2 drops of thoroughly shaken latex antigen, mix and spread over an area of 1″ in diameter.

4. Rock the slide slowly and read the test for agglutination at the end of 2 minutes.

5. Absence of agglutination at the end of 2 minutes indicates pregnancy (Fig. 1).

Ovarian Hyperaemia Test

Two ml. of test urine sample were injected intraperitoneally in 3-4 weeks old immature rats. After 24 hours, rats were sacrificed and ovaries were observed for corpora haemorrhagica (Zondek *et al* 1945).

Observations

The results of tube tests are summarised in Table I.

Out of 34 samples, 17 gave a positive reaction. Clinical criteria confirmed pregnancy in 14, one was a case of abortion and in one case there was probably pregnancy with ovarian cyst but no follow up was available. There was one false positive test.

TABLE I

Results of tube immunological test

Days from last menstruation	Total	Positive test	False positive	Clinical findings in retrospect	
				Pregnancy	Abnormal pregnancy
40 –50	11	7	..	7	..
50 –60	8	4	1	3	..
60 –70	7	2	..	2	..
70 –80	..	..	..	..	..
80 –90	2	2	..	1	1 (Abortion)
90 onwards	6	2	..	1	1 (Ov. Cyst + Pregnancy)

TABLE II

Results of slide test for pregnancy

Slide test results	Clinical findings in retrospect		
	Pregnancy	Abnormal pregnancy	Missed periods (No pregnancy)
Positive	95	7	..
Negative	..	1 (ectopic)	42
False negative	5	..	..
False positive	..	..	..

Slide test for pregnancy was carried out in 150 samples. One hundred and two samples gave a positive reac-

***Ortho gravindex kit for immunological slide test.*

tion for pregnancy. Seven of these samples were found to be from cases of abnormal pregnancy (3 hydatidiform mole, 2 choriocarcinoma, 2 abortions). False negative test was observed in 5 samples. Forty-three samples were negative, confirmed clinically in all cases except one, which was a case of ectopic pregnancy.

Table III shows that out of 5 false negative tests, 4 samples were from early pregnancy (42 to 50 days) while one was from late pregnancy (150 days). Out of the 7 abnormal pregnancy cases showing positive reactions 3 had hydatidiform mole, 2 were cases of threatened abortion and 2 of choriocarcinoma.

Table IV gives an evaluation of slide immunological test and biological tests for pregnancy. Rat test was carried out in 101 samples only. Of the 5 false negative slide tests, 3 were from early pregnancy (50 days gestations). The rat test was also negative in these samples. A week later both tests became positive in these subjects. In the 4th case (42 days gestation) the slide test was negative while the rat test gave positive result. A week later the slide test became positive. In the 5th case the gestation was of 150 days and both slide and

TABLE III

Slide immunological test related to period of pregnancy in 150 urine samples

Days from last menstruation	Total	Positive test	False negative	Clinical findings in retrospect	
				Pregnancy	Abnormal pregnancy
40—50	36	16	4	20	....
50—60	32	21	....	21	....
60—70	12	9	....	9	....
70—80	23	19	....	18	1 (Hyd. mole)
80—90	18	11	....	8	3 (Hyd. mole1) (abortion 2).
90—100	5	4	....	4	....
100 onwards	22	20	1	20	1 (Hyd. mole)
Unknown	2	2	....	....	2 (choriocarcinoma).
Total	150	102	5	100	7

TABLE IV

Evaluation of slide immunological test and biological test for pregnancy

Days from last menstruation	Slide Test		Rat Test	
	Positive	False negative	Positive	False negative
40—50	12	4	13	3
50—60	19	....	19	....
60—70	9	....	9	....
70—80	19	....	19	....
80—90	11	....	11	....
100 onwards	20	1	20	1
Unknown	2	....	2	....
Total	96	5	97	4

rat tests gave false negative results which is understandable.

Comments

It is believed (Islami *et al,* 1964 and Noto *et al,* 1964) that the level of H.C.G. goes on rising from about 42 days (from the last menstrual period) and reaches its peak between 60-90 days and then gradually tapers down.

The presence of H.C.G., however, has been detected by some observers (Hon. 1961) even before the first missed period (10 days after ovulation) by immunological test. In our laboratory, as discussed already, this slide test was not so reliable before 50 days from the last menstrual period. The specific gravity and pH had no influence on the results of slide immunological pregnancy test.

It is not possible to compare the accuracy of slide test over the tube test as fewer samples were tested by tube test. But it may be mentioned that the slide immunological test offers several advantages over other immunological pregnancy tests. It is quick and very easy to perform, and it does not require elaborate laboratory set up. No discrepancy in results was marked by the use of the different batches of reagents.

In our series there were no false positive slide test or false positive rat test. The reason for 5 false negative tests has already been commented upon. This was probably due to very low levels of hormone in the early days of gestation in such cases. Out of these 4 cases, 2 cases went to full term while 2 aborted. The 5th false negative case was in the later stage of pregnancy (150 days) when H.C.G. had probably returned to very low levels.

Summary

1. One hundred and eighty-four urine samples were tested for pregnancy. Thirty-four samples were tested by latex tube method and 150 samples were tested by slide method. Rat ovarian hyperaemia test was carried out in 101 samples to confirm pregnancy slide test. Specific gravity and pH test was recorded in 100 samples.

2. Slide immunological test for pregnancy was found to be a reliable and rapid test which gives result within 3 minutes and is technically very simple to perform.

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Fig. on Art Paper II