EVALUATION OF PREGNANCY TESTS

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The importance of diagnosis of pregnancy in its early stages is accepted. Every obstetrician and general practitioner is, some time or other, confronted with the problem of correct opinion in patients with history of amenorrhoea, whether it is due to pregnancy, hormonal imbalance, genital tuberculosis or just delayed. The urgency of exclusion of ectopic pregnancy in cases of acute abdomen is of great significance to every surgeon.

Aschheim and Zondek, in 1929, described for the first time a biological test of pregnancy. The test was based on gametokinetic response of ovaries of immature female mice on injecting urine from suspected pregnant women. The results are obtained as enlarged, hemorrhagic Graffian follicles in ovaries, the well known "blut punkt" appearance.

In spite of its remarkably high accuracy, and hence world-wide acceptance, the test has some major drawbacks in its applicability on a large scale. The chief objection was its delay in reading of the results, which could not be available to the physician before 5 days. The urgency of immediate diagnosis in cases of disturbed pregnancy, like ectopic pregnancy, still remained unfulfilled. Further, as each test requires five mice, a well maintained large animal house was essential.

The well known "Freidman test" was introduced by Freidman and Lapham in 1931, using female rabbits as test animals. Though the successful utility of this animal has been confirmed by many workers like Schneider, Ducy and Albert, yet the main disadvantage of injecting large volumes of urine intravenously in the ear vein remained unsolved. Moreover the frequent toxic action of urine on rabbits has limited its use as the test of choice for routine use.

Kelso put forward a two mice technique modification of Aschheim Zondek test with the added advantage of shortened test time to 30 hours. This remarkable achievement induced a wide interest in such an investigative procedure and soon successful reports appeared in literature regarding the possible use of other animals.

The efficient use of female rats as test animals was first introduced by Eberson an Silverberg in 1931. This test is based on the same principle as Aschheim-Zondek test, with the exception that 2 rats are employed in each test and the results are obtained within 2-6 hours. Conflicting reports have however appeared in the

literature from time to time regarding its reliability (Frank, Walker and Walker, Kelso, Salman *et al*, Kupperman and Greenblatt).

It was not until 1947 that Gali Mainini described the use of male South American frog 'Rana Pipiens', and male American toad as the test animals. These tests are based on spermatogenic release in the tests on injection of urine containing increased level of gonadotropic hormones. These tests have manifold advantages over the previous ones in being relatively more simple, dependable and economical. The results are obtainable within 2-3 hours. Moreover the same animals are re-utilised for 4-6 times before being discarded, and they can be maintained on only water with no other food requirements. Robbins and Parker, Wiltberger and Miller, and Brody have been critical of this test, due to individual sensitivity variation and seasonal variation of the test animals.

The biological tests described so far, demand the use of some animal e.g. mice, rats, frogs or toads, each one of which has one or the other disadvantages. The demand for a more simple and less time consuming test was met successfully by Richardson in 1952. He introduced a chemical test based on the detection of free estrone in urine of pregnant females. The test requires but few cheap chemicals, it is easy enough to be performed by a technician, and above all, it just takes 10 minutes for its completion. Hence its use, as an emergency measure in the operation theatre or as a screening test in an obstetrical clinic, can not be overemphasized. However, the lower percentage of accuracy has been strongly criticised by many workers e.g. Merkel, Fischer, Benjamin and Segaloff and Rapp. They have all remarked on its high level of false positive results. One interesting observation noticed by every worker is its high accuracy in ectopic pregnancy but no explanation so far has been forwarded for this.

Recently an extremely simple cytological test of pregnancy has been described by Roland. This is based on cyclic changes in cervical mucus smear due to hormonal variation.

The present work was undertaken to evaluate few of the important groups of pregnancy tests, e.g. biological, biochemical and cytological, to establish a routine laboratory diagnostic test of pregnancy, a test which is accurate, simple, cheap, rapid as well as dependable. The following groups of tests were performed in each patient.

1. Aschheim-Zondek Test employing immature mice.
2. Galli Mainini Test employing frogs.
3. Galli Mainini Test employing male toads.
4. Richardson's chemical test.
5. Roland's cystological test.

Material and Methods

The patients were selected from obstetrical out-patients and antenatal clinic. They were listed in one of following 4 groups:

1. Early pregnancy cases: In this were included women giving history of amenorrhorea of less than 16 weeks' duration, the menstrual cycle being previously normal.

2. Late pregnancy cases included patients giving history of amenorrhoea of more than 16 weeks duration.

3. Early disturbed pregnancy cases, including abortion, ectopic pregnancy, vesicular mole.

4. Control cases: This consisted of 50 female patients, having amenorrhoea as a symptom of lactational period, functional bleeding, tuberculosis of genital tract or menopause.

Few urines from male patients were also subjected to these investigations.

Following preliminaries were observed before the actual tests were performed:

1. The patient was asked to bring her first morning specimen of urine to the laboratory.

2. Detailed personal history taken of the patient regarding duration of amenorrhoea, previous pregnancies and her usual menstrual cycles.

3. Thorough abdominal and vaginal examination was undertaken.

4. Cervical secretion was obtained on a glass slide.

Standard techniques as described by the authors were used in the performance of the various tests.

Results

260 cases were studies as follows:

Group 1. Normal early pregnancy .. 116
Group 2. Normal late pregnancy 60
Group 3. Disturbed pregnancy including cases of threatened abortion, incomplete abortion, ectopic gestation in fallopian tubes and vesicular mole. .. 24
Group 4. Female and male control cases .. 60

Group 1: 116 cases were investigated. The results are summarized in the following table.

Group 2: Under this group were included cases coming with amenorrhoea of more than 16 weeks' duration. In all 60 patients were investigated. An interesting observation was made regarding the spermatic response in toads and frogs. As the period of amenorrhoea increased the number of sperms per high power microscopic field decreased. In some cases hardly 1-2 sperms in whole drop of urine could be noticed. The results are given in the following table:

TABLE I.

Type of Tests	No. of Tests	Correct Positives	False Negatives	Percentage of Accuracy
Frog Test ..	116	113	3	97.43
Toad Test ..	38	37	1	97.37
Mice Test ..	31	31	0	100
Chemical Test ..	116	109	7	93.97
Cervical Smear Test ..	116	116	0	100

TABLE II

Type of Tests	No. of Tests	Correct Positives	False Negatives	Percentage of Accuracy
Frog Tests ..	60	56	4	93.34
Toad Test ..	23	22	1	95.65
Mice Test ..	13	12	1	92.46
Chemical Test ..	60	55	5	91.67
Cervical Smear Test ..	60	60	0	100

Group 3: Under this group were included cases of abnormal early pregnancy viz. threatened abortion, incomplete abortion, ectopic pregnancy and vesicular mole. The findings are tabulated as follows:

TABLE III

Type of Tests	No. of Tests	Correct Positives	False Negatives	Percentage of Accuracy
Frog Test ..	24	20	4	83.4
Toad Test ..	6	5	1	83.4
Mice Test ..	3	3	0	100
Chemical Test ..	24	20	4	83.4
Cervical Smear Test ..	24	19	5	71.7

The cervical mucus smear examination proved inconclusive in those cases where bleeding per vaginam was present. The presence of red blood cells obscured whole pattern of the smear.

Group 4: Under this group 60 control urines were tested and surprisingly enough no false positive result was obtained with any of the tests. This suggests the certainty of diagnosis in positive results though a negative result does not necessarily exclude pregnancy.

The following gives the composite analysis of results of all the tests:

TABLE IV.

Final diagnosis	No. of Tests	Correct Positives	Correct Negatives	False results	Percentage of Accuracy
Early normal pregnancy	116	113	0	3	97.5
Late normal pregnancy	60	56	0	4	93.4
Disturbed pregnancy	24	20	0	4	83.4
Control cases	60	0	60	0	100

Discussion

1. *Biological tests*

Although these tests essentially differ from each other as regards the choice of animal and technique of injection, all are based on one common principle of gametokinetic response of ovaries or testes upon injection of urine containing considerable level of gonadotropic hormones.

A. Male Frog Test:

According to the method described by Galli Mainini the test was found to give an overall accuracy of 95.77 per cent in 260 cases. However, a marked difference in results was seen according to the duration of amenorrhoea. In early months of pregnancy the spermatozoa were plenty, actively motile and appeared within ½-1 hours in urine drop, but in later months of pregnancy especially after the 32nd week, this response was meagre and often completely absent in a few cases. In the first group correct results were obtained in 97.34 per cent of cases, while in the latter group in only 93.34 per cent cases. Very few workers have observed this difference in the reactivity of frogs. Wiltberger and Miller have reported 50 per cent false negative results in the second and third trimester of pregnancy. Bienierz noticed a fall of 22.4 per cent accuracy in late months of pregnancy. Bodine showed that the test gave 100 per cent accuracy in the first trimester, 83 per cent in the fourth month, 75 per cent in the fifth month and only 36 per cent in the sixth month. Gupta and Mangalik, however, obtained 97.5 per cent accurate results in 37 cases of late pregnancy.

Abnormal state of pregnancy is another factor of importance which influenced the results. In cases of threatened abortion, dead foetus, and ruptured ectopic pregnancy, the contact of foetal to maternal circulation is not intact, hence the level of circulating gonadotrophic hormones is not high enough to register a positive response. It is in these cases that a negative result is often encountered.

The test in frogs is highly specific in that no positive response was elicited by injection of nonpregnant urine. It is obvious then, that a positive response speaks only of increased excretion of gonadotropic hormones in urine, but a negative response does not necessarily exclude the same.

Confusing reports have appeared in literature regarding seasonal variation in frogs during the summer months (Reinhert, Brody, Robbins and Parker). The present series of tests were carried out in the winter months and as such no opinion can be expressed in this aspect.

B. Galli Mainini's Test on male Toads:

This animal was reported on by various workers (Galli Mainini, Forman *et al,* Blanchard and Bretto, Jones and Jones) and was found to be a very reactive and reliable one. The observations nearly ran parallel with male frog tests. But since the toads are not conveniently obtained throughout the season and since it is sometimes very difficult to differentiate the sex, male frog was accepted as a superior test.

C. Kelso's modification of Aschheim-Zondek Test:

50 cases were investigated and only 1 negative result was noticed in late pregnancy. The results are in agreement with previous workers (Landgrebe and Hobson, Keegan, Marsters *et al).* The test is extremely reliable but since it is expensive and time consuming it is proposed

that its use should be restricted, as a confirmatory test in those cases where doubtful or negative response is obtained, in a case of suspected pregnancy, with one of the other simpler tests.

II. Chemical Test of Pregnancy

This test originally devised by Richardson in 1951, has suffered serious criticism at the hands of different workers in spite of its easy performance and rapidity of response. In the present series inferior percentage of results were observed as compared to the biological tests and the findings are in complete accordance with other workers (Merkel, Fischer and Mc Colgan, Rapp).

The test is outstandingly simple from every point of view and hence its use as a screening test in antenatal clinic and as a routine measure is recommended. A positive result definitely suggests a pregnant state, while a negative result indicates a repetition of biological tests to exclude pregnancy.

III. Cervical smear test of Roland.

This extremely simple test was found to be a useful and reliable one, in that it gave very accurate results. The cellular pattern of crystallization in the smear is diagnostic of pregnancy when a patient has missed a regular period. The only other condition which can give rise to this smear is luteoma or corpus luteum cyst which is relatively rare. The one disadvantage of this test is its inaccuracy in cases which come in for bleeding per vaginam, as the presence of red blood cells completely obscures the pattern.

Summary and Conclusions

1. A preliminary work on few important tests of pregnancy is presented to assess and evaluate the practical utility of each.

2. Aschheim-Zondek Test is recommended as the most reliable single test of pregnancy in that it is very sensitive to even low levels of chorionic gonadotropins and it never gave false positive results.

3. Male frog and male toad tests introduced by Galli Mainini have proved equally reliable and have manifold advantages over the A.Z. test, in that these animals are cheaper, they can be utilised a number of times and give the response within a few hours of injection. Their slightly lower percentage of accuracy is a minor handicap as a repetition of test in mice in suspicious cases can be undertaken.

4. Chemical test of Richardson is recommended as a routine screening test in every out-patient department, as it is easy to conduct and requires but few inexpensive chemicals. A positive result supports pregnancy. If the result is negative, in a case where clinical evidence favours pregnancy, the biological test should be performed.

5. Cervical mucus smear test looks a reliable test but it requires further trial, before it can be accepted as an effective investigative procedure. The number of controls is too little to permit formulation of definite opinion at this stage.

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