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Laboratory Diagnosis of Pregnancy

R. L. Reese, '45

Laboratory methods for early diagnosis of pregnancy have found their greatest application in the human where clinical symptoms of the condition are not manifest until approximately 6 weeks following conception. With domestic animals, the mare stands alone as the animal to which laboratory tests may be applied. Diagnosis of pregnancy by rectal palpation in the mare is desired over laboratory methods because it is faster, more accurate and can be applied earlier in the gestation period. However, laboratory tests are employed on those mares whose delicate rectal mucous membranes may not tolerate manipulation. In highly bred mares with nervous temperaments it may be impossible to perform rectal palpation due to incooperation and possibility of abortion.

Veterinarians who have added laboratory facilities to their practice could well consider the possibility of conducting pregnancy tests for the local physician and, when indicated, for his own equine patients. A discussion of the pregnancy tests best adapted to the veterinarian's laboratory will be made as well as mention of other tests which may be of interest. These tests include the mucin test, the chemical test (Cuboni’s test) based on color changes in urine, the colostrum test, and animal injections of urine or blood serum from suspected pregnant females which include—Hogben’s Toad Test, the Ascheim-Zondek Test, and Friedman’s modification of the Ascheim-Zondek Test.

The tests which make use of pregnancy urine are based upon the action of anterior pituitary-like hormones found in the urine of pregnant females. These hormones stimulate specific changes in the gonads of experimental animals when injected in adequate quantities. The APL hormones may reach a concentration sufficient to produce a positive test for the human as early as 7 days after conception. The same is not true of the mare in which 150 days of fetal growth are required to furnish a satisfactory hormone concentration in the urine. Blood serum must be used to determine pregnancy in the mare if the test is to be run 40 to 150 days after conception.

First Pregnancy Test

One of the first pregnancy urine tests used was the Ascheim-Zondek test. Immature female mice from 3 to 4 weeks of age were used in the procedure of this particular test. A series of six injections of 0.5 cc. per dose of pregnancy urine over a period of 48 hours is administered to each of several mice. The mice are sacrificed 96 hours after the first injection and examined both macroscopically and microscopically. A positive reaction to prove pregnancy is indicated by gross enlargement, irregular outline and hyperemia of the ovaries. The accessory genital organs are also grossly enlarged and congested in comparison to those of the control mice. Microscopic section of the ovaries shows many large follicles in various stages of maturation.

Both extra-uterine and intra-uterine pregnancies result in positive Ascheim-Zondek tests as does the hydatid mole or chorion-epithelioma because this test is merely an indication of the presence of living tissue derived from a fertilized
ovum. As long as the ovum or any of its proliferating elements are alive, the test gives a positive reaction. Urine tested some time after abortion or intra-uterine fetal death will give a negative test. The positive reaction appears during normal pregnancy but disappears several days following parturition.

The Ascheim-Zondek test has several disadvantages, two being the long period of time required to obtain a diagnosis and the difficulties encountered in obtaining and maintaining enough mice of the correct age, sex, and maturity. One big advantage of the test, however, is its high rate of accuracy.

**Modifications**

Many modifications of the original Ascheim-Zondek test have appeared since its introduction. Some of these modifications are being used commonly, but still others are yet in the experimental stage. Hogben’s *Xenopus laevis* toad test, recently developed, makes use of injections of suspected urine into the dorsal lymph sac of the toad. The extrusion of hundreds of macroscopic eggs occurs in 6-15 hours if the urine contains sufficient amounts of APL hormone. However, it does not give graded results and hence is not an indication of the hormone concentration. This test has not been used to any great extent in the past few years because of the difficulty encountered in shipping the toads during wartime. The injection of capons with pregnancy urine has been practiced also, but has not been widely used as yet.

**Rat Test**

One modification of the Ascheim-Zondek technic is the 24-hour rat test. This test involves the use of 22 to 40-day-old immature female albino rats as the experimental animals. The urine used is collected from the suspect on the morning following a day of restricted fluid intake. After collection, the urine is concentrated and detoxified. If the sample is to be shipped, 1 drop of tricresol (lysol) per ounce of urine or 4 grains of boric acid crystals per 100 cc. of urine may be used as preservatives which will last for at least 6 days. The urine is stored at 37° C., as a temperature of 60° C. or higher will inactivate the hormone. A 24 gauge needle about 1½ inches long is used for the subcutaneous injection on the dorsal surface of the neck. Two rats are injected with 1 cc. doses 3 times a day for 1 day, and 2 more rats are injected with 0.5 cc. doses 3 times a day for 2 days. The first 2 rats are killed 24 hours after the first injection and examined for hyperemia and enlargement of the ovaries. For a positive reaction, both changes must be present. The next 2 rats are killed 72 hours after the first injection of 0.5 cc. of urine, and they act more or less as a check on the 24 hour test. The advantages of this modification are its rapidity, convenience and economy. Rats are easy to procure, breed and accommodate in large numbers.

Of all the animal injection tests now used to determine pregnancy in humans and horses, perhaps the one of the most practical importance to the veterinary practitioner will be Friedman’s modification of the Ascheim-Zondek test. This test was formulated by Friedman and Lapham in which they utilized isolated rabbits of not less than 17 weeks of age and not less than 1500 grams in weight instead of mice. The rabbit has no menstruation period; hence ovulation usually occurs only at the time of coitus. If a rabbit is to be used as the experimental ani-

![Positive Ascheim-Zondek test showing ovaries of rabbit 48 hours after injection of urine.](image)
mal to be injected, she must be isolated for a period of approximately 12 days. The test calls for an initial intravenous injection of 5 to 7 cc. of suspected urine or blood serum into the ear vein of the isolated rabbit. This dose is followed in 24 hours by an additional 3 cc. injection at room temperature. It has been found by some workers that an initial dose of 9 to 12 cc. injected slowly intravenously will get correct results in rare cases when the smaller dosage fails to detect a pregnancy. The marginal ear veins are the veins of choice for the injections instead of the larger middle ear vein because the latter tends to slip away from the needle. The rabbit is sacrificed and examined 48 hours after the first injection. Fresh corpora lutea or corpora hemorrhagica are indicative of a positive test. The ovaries of the rabbit are much larger than those of the mouse; hence this test is comparatively easy to read macroscopically.

**False Reactions**

Certain individual difficulties may result in false reactions to this test. The serum from some mares has the characteristic of causing no reaction in the rabbit's ovaries in spite of the fact that they are normally pregnant. One of these mares was tested 3 different times during pregnancy and she never did show a positive reaction. She completed her gestation period, however, and gave birth to a live foal. Human urine sometimes contains a toxic factor which hinders a correct reaction in the rabbit. The samples from one human suspect tested gave severe reactions in 3 rabbits; the true Aschheim-Zondek test proved this individual pregnant.

Restraint of the rabbits during the injection is an important but commonly overlooked factor in the success of the Friedman modification. Many times the injection of urine causes the rabbit to struggle, thus pulling the needle from the vein. A small box having a sliding lid with a hole in it just large enough for the rabbit's neck may prevent the difficulty.

Some men prefer to make the injections subcutaneously, but the intravenous method has been preferred because it is thought to be more accurate than the subcutaneous procedure.

Tests not involving the use of blood serum or urine from the suspected female are the mucin test and the colostrum test. The mucin test involves the microscopic study of smears from the vaginal epithelium. The presence of globules of mucus, columnar epithelial (pregnancy) cells in which the cytoplasm stains lightly, and few other cells in the smear indicates pregnancy. The test has thus far been used mainly for the diagnosis of human pregnancy.

The veterinarian who finds a need for pregnancy diagnosis within the sphere of his practice may consider the possibility of performing such in his own laboratory. The equipment needed to run the Friedman modification of the Aschheim-Zondek test is not extensive. Readily cleaned individual cages are needed for the rabbits. The rabbits may be purchased from a commercial producer or local establishment and must be of a type satisfactory for the test. A 10 cc. glass syringe with a 22 gauge ½ in. needle is needed to inject the urine. A suitable box should be obtained for restraining the rabbits during injection.

It must be emphasized that any human pregnancy tests should be made in conjunction with medical doctors and that the results of all tests are confidential.

**BIBLIOGRAPHY**


Normally, 500 birds should be allowed 5-10 acres with ½ to ¼ acre for each move.