

Methods of Pregnancy Diagnosis in Mare- A Review

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Review Article

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Article History

Received: 17.03.2018

Accepted: 03.04.2018

Published: 30.04.2018

DOI:

10.36347/sjavs.2018.v05i04.001



Abstract: Methods of pregnancy diagnosis on mare classified into visual, clinical and laboratory methods. Visual methods of pregnancy diagnosis not accurate than clinical and laboratory methods. Laboratory methods with hormonal assay give results when early pregnancy, but false positive results are more. Ultrasonography gives good results when compared to other chemical and biological tests. Ultrasonography rule out the fetal death and twin pregnancy also at early stage.

Keywords: Biological methods, Chemical test, Mare, Visual methods.

INTRODUCTION

Early pregnancy diagnosis in equine species is very important, because seasonality in breeding. If non-pregnant mare found on examination, it should be teased and try to get them pregnant in the same season of the year. Moreover early diagnosis of pregnancy in mare useful when, twin pregnancy occur. It should be terminated, unless gestation related complication occur. There is a need to educate the owners to get their animals checked for early pregnancy diagnosis. Laboratory methods are used in past and slowly developing in recent year with advances. Earlier the pregnancy diagnosis performed, the more profitable for the stud owners [1].

METHODS OF PREGNANCY DIAGNOSIS

A wide variety of approaches have been used and developed during past and recent years, some of which have advantages and disadvantages over other methods.

I) Visual methods:

- Non-return to estrus (Cyclic),
- Increased in size of abdomen,
- Udder development, d) Foetal movement-Externally,
- Vaginal discharge

II) Clinical methods:

- Vaginal examination,
- Recto-genital palpation
- Ultrasonography
- Abdominal ballotment

III) Laboratory method:

- Milk-Progesterone assay,
- Chemical test,
- Biological tests

I) Visual Methods:

- a) Non-Return to cyclic:** Mare is does not return to estrus after last mating the owner usually thinks that the animal has become pregnant. This happens during pregnancy, due to the early embryo or conceptus inhibits the lysis of the corpus luteum (Maternal recognition of pregnancy) and thus, prevents the animal from returning to cyclic. However, some times the animal does not come to cyclic because of persistent CL due to pathological condition of the genitalia like mucometra, pyometra, hydrometra, ovarian cyst [1]. Moreover, equine species are long day seasonal breeder may not return to estrus when mating is done during the end of the actual breeding season, because the season was over. Estrus detection methods used for seasonal breeder need to be properly designed and equipped so as to make efficient use of non-return to estrus as a method of pregnancy diagnosis in

these species. Most of the time estrus detection device not giving proper results when mares are in stall fed farm condition. Therefore, non-return to cyclic is an unreliable tools for pregnancy diagnosis in equine species. *Heat signs observed in gestation termed as Post- conception heat.* The demonstration of heat signs generally requires the presence of trained teaser stallion or androgenised gelding (Castrated horse). This type of practice should be done after 16 days of mating (14-16th days of luteal phase considered as MRP in mares) and continue for a consecutive 6 days (because breed variation). Following reasons are contributed for false positive results, 1) *mare has in silent heat*, 2) *lactational anestrus*, 3) *prolonged luteal phase may associated with early embryonic mortality; this condition reffered as Pseudo-pregnancy.*

- b) Increased in size of abdomen- from 6th month onwards.
- c) Udder development- last trimester of pregnancy
- d) Foetal movement externally visible on right side of abdomen in late gestation.
- e) Vaginal discharge- 6-7th month of pregnancy thick mucus discharge from vagina.

II) Clinical Methods:

a) Vaginal Examination: Vaginal examination done by using *Russian type Vaginal speculum*; however manual exploration can be used. The characteristics of vaginal mucosa is pale to pink, the mucus during diestrus is scanty and sticky, and cervix size apparently small in size and tightly closed with cervical seal of pregnancy. False positive also occur as a result of prolonged luteal phase of cycle and pseudo-pregnancy.

b) Recto-genital Palpation: The early diagnosis of pregnancy in the mare has its own significance characteristics changes in uterus, ovary and cervix. A limited breeding season in some equine breed registries like through breeds necessitate the non pregnant mares are diagnosed early as possible and steps be taken to breed the mare again in the same breeding season without fails. Diagnosis of an early pregnancy by trans rectal palpation is one of the easiest, cheapest and accurate means for experienced veterinarians. However, a diagnosis at day 18-20 should be re-examined and confirmed at day 45-60 because of a delayed implantation of the equine conceptus and chances of early embryonic mortality and reabsorption of embryo occurs.

Table-1

1.Palpation of Ovary	Both ovaries should be examined properly. Due to follicular development both ovaries are enlarge from 18 to 40 days, but CL is not palpable. Secondary CL is evident due to ovulation, lutenization at 40 to 120 days. Follicular activities becomes decreases from days 120 to term, consecutively the ovaries become small and inactive. Location of the both ovaries either pregnant or non-pregnant, similar up to 60days of pregnancy. At 3 to 5 month of pregnant both ovaries are near to each other and closer to the pelvic floor, moreover uterus or other uterine structure not palpable at this time. 5 months onwards ovaries are generally hide under the broad ligaments.
2.Palpation of Cervix	Pregnant animal cervix becomes tightly closed, firm, elongated as early as 16 to 18 days after ovulation. Between 16 days above the cervix can be with experience palpated on the pelvic floor as a rigid firm structure.
3.Palpation of Uterus	a) Increase in uterine wall thickness and marked tone started at days 15 to 16 and end at 48 to 55th days [2]. b) At 25 to 28 days small bulge palpable in the base of one of the uterine horn at the junction between uterine horn and body. c) At day 30 to 35 palpable of conceptus in most mares. d) At 42 to 45 days the conceptus occupying half of gravid uterine horn, moreover at days 60 nearly entire gravid horn and body of the uterus occupied by conceptus.
4.Palpation of Fetus	a) At 40 days- active fetus palpable b) At 70 days- Mobile fetus palpable. Most probably from 3rd month onwards fetus can be palpable per rectally. From 5th month to 7th month of gestation fetus not able to palpable (Varies with breed characteristics). In this situation location of the uterus and palpation of ovary as guide for pregnancy diagnosis.
5.Location of the Uterus	a) Upto 90 days the uterus is located at the pelvic brim. b) At 100-200 days- Cranial to the pelvic brim, moreover in the abdominal cavity [2]. c) At 5 to 7month- Uterus positioned into lower abdomen (Horns are perpendicular to the dorsal cranial aspects of the uterine body). d) Above 7 to 8 months- Fetus easily palpable per rectally.

c) Ultrasonography: The methods of detection of pregnancy using ultrasonography (USG) have been

described for various species in sufficient detail by Goddard [3].

- The earliest ultrasonographic finding for pregnancy in most animals is the appearance of *anechoic fluid* within the uterine lumen structure.
- This fluid goes on increasing to a stage of pregnancy.
- The embryo proper becomes visible as a hypoechoic clear structure freely floating within this amniotic fluid, moreover gestation advances

progressively the fetal structures become more densely visible along with the fetal membranes. The viability of a growing fetus is detected when the fetal heart beat becomes visible as a *hypoechoic continuous flickering structure*. The most appropriate time for pregnancy diagnosis using ultrasonography with high accuracy in mare [4] appears to be day 24-25 after mating or AI.

Table-1: Trans rectal linear array probe is used for pregnancy diagnosis, at most probably 5.0 to 7.5 MHZ frequency

Days of pregnancy	Early Ultrasonographic findings	References
10-16	<i>Foetal fluid (Anechoic)</i>	Allen and Goddard, 1984 [5]; Ginther, 1986 [6] ; Sertich, 1997 [2]; Pycocock, 2007 [4]; Holder, 2007 [7]
24-25	<i>Foetal heart beat (Flickering)</i>	
20-22	<i>Cotyledons/Allantois</i>	
20-22	<i>Fetus structure</i>	
60-70	<i>Foetal sex determination (Genital tubercle)</i>	
40-45	<i>Foetal movements (Moving from the probe axis)</i>	

d) Abdominal ballotement: In mare abdominal ballotement technique is performed by placing the right hand fist over the lower right abdominal wall and pushing it in an intermittent manner slowly in a dorsal medial direction deeply. The fetal parts can be felt as a hard solid object floating in fluid. This is usually possible in lean mares after the 7th month of gestation onwards to term.

III) Laboratory Methods: Laboratory diagnostic tests are evolved for pregnancy diagnosis in mares. They are indirect methods of pregnancy evaluation, and utilize qualitative/ quantitative measures of reproductive hormones at specific stages of pregnancy after last artificial insemination [1] or natural mating, or detect conceptus/ embryo specific substances in maternal body parts or body fluids (Serum, urine) as indirect indicators of the presence of a viable pregnancy. Moreover, none of the diagnostic methods are developed so far in farm animals are as accurate as is the detection of human chorionic gonadotrophins (hCG) in pregnant human females. The currently feasible methods are briefly described.

a) Serum and Milk progesterone assay: The corpus luteum formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for a reasonable time period in some species. Non pregnant mares not returning to cycle and pregnant mares in which embryonic death occurs at a later time can both give false results. Moreover, in mares, sheep, goats, buffaloes, camels and sows assay of plasma or milk progesterone estimation is not very accurate method for diagnosis of pregnancy [8-12]

b) Chemical Test for pregnancy diagnosis:

i) Cuboni's Test: Cuboni test was first developed by Cuboni [13]. The test is performed in the mare for pregnancy diagnosis through assay of *conjugated estrogens* present in urine of pregnant mares.

- Take 15 ml of urine in conical flask and add 3 ml of concentrated hydrochloric acid
- Heated in a water bath for 10 minutes and then cooled under a tap.
- To this 18 ml of benzene is added and shaken vigorously for half a minute.
- 4. The supernatant is collected in another test tube and add 3 to 10 ml of concentrated sulfuric acid and the mixture heated in a water bath at 80°C for 5 minutes,
- Then allowed to cool the test tube. Keep the test tube under fluorescent light.

Impression: A positive (pregnant mare) test is indicated by the appearance of a dark, only green fluorescent color in the lower sulfuric acid layer A negative (non-pregnant mare) is characterized by absence of fluorescent color and presence of a brownish color.”

The cuboni test is only effective after 150 days of pregnancy, and also used for predicts the fetal viability.

ii) Mouse Test:

- The serum or urine collected from pregnant mares
- It injected to ovariectomised mouse or rats.
- It would induce vaginal edema, appearance of cornified or anucleated cells (estrus predominant cells) and mucus discharge from vagina due to presence of estrogens in the pregnant mare’s serum or urine [14].

iii) Phenolsulphonic acid Test: Phenolsulphonic acid reagent react with urinary estrogen gives color changes. The final reaction gives a pink to cherry red color if mare pregnant [15, 16]. Phenolsulphonic acid tests are 70-80% accurate when done between the 120 to 250 days of gestation.

iv) Mucin test/ Kurosawa test:

Vaginal mucus from a pregnant mare showed *dark staining columnar epithelial cells* (they are called pregnancy cells) [17] and known to have shown that an efficacy of 94% from day 70 to end of pregnancy [18, 19].

C) Biological Test: Biologic tests were developed for the detection of eCG: *Aschiam Zondek Test, Friedman test (rabbit test), Frog or toad test.*

i) Aschiam Zondek test (A-Z Test):

Serum separated from mares and it is injected to immature mice [18] results were interpreted.

- The serum about 0.5 ml SC daily for 2 to 4 days or 5 ml intra peritoneal injected to 2 to 3 rats (22 days of age)
- Rats are killed. Criteria 72 hr when injected intra peritoneal and 96 to 120 h later when injected subcutaneously

Impression: positive for pregnancy is indicated by the presence of multiple small sized corpora haemorrhagica on the ovaries and uterine edema in rat. The test was considered to be 90 percent accurate in nature when performed between the 60 to 100 days of gestation in the mare.

ii) Friedman Rabbit test:

- Select approximately 14 to 20 weeks age old rabbits.
- Serum from test mare is injected to rabbits at 2 ml (IV)
- Kept separately and laparotomy performed 24 hour after serum injection.

Impression: A positive for pregnancy is indicated by the presence of corpus haemorrhagicum and uterine edema in rabbit.

iii) Toad test:

Toads like *Bufo valliceps* or frog like *Rana Pipiens* are used in this test. The principle of this test is that the sperm cells are emitted by toads/frogs only when stimulated with female frogs or gonadotrophins hormones.

- Two male toads are taken for test and their cloaca is cleaned with normal saline and examined for presence of spermatozoa.
- If no sperms are present in the cloaca, 1 ml of test serum from a mare is injected into the dorsal lymph sac of the 2 male toads thrice at 1 hour interval.
- Then cloaca is examined for the presence of the sperms after 6 hours of last injection.

Impression: Positive for pregnancy is indicated by the presence of sperms in the cloacal region within 1 to 6 hours after the last injection [20, 21].

CONCLUSION

Even though advanced kits facility techniques developed, the veterinarian depends on Trans rectal palpation of pregnancy diagnosis only. That is the easy and cheapest methods for early pregnancy diagnosis in mare. Because kits are gives false positive results when fetus dies after formation of endometrial cups. As per equine concern sensitive diagnostic methods are necessary to rule out the early pregnancy. *Moreover ultrasonography gives good results when compared to other chemical and biological tests.* Because its rule out the fetal death and twin pregnancy also at early.

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