

## PREGNANCY DIAGNOSIS IN A 4-YEAR-OLD MARE: A CASE REPORT

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### ABSTRACT

This is a report of pregnancy diagnosis, conducted at an early stage, and subsequent stages on a 4-year-old mare that sustained some injuries. The methods used for pregnancy diagnosis include rectal examination and hormonal assays using a modified Aschheim-Zondek (A-Z) test. Two guinea pigs weighing 356 g and 351 g, respectively, were used. The first one was inoculated with serum (1.5mls) obtained from the blood of the mare and sacrificed two days post-inoculation. The second guinea pig was inoculated with 1.5 mL of serum after 10 days and sacrificed two days post-inoculation. The mare's bruises were managed with disinfectants, NSAIDs, Gentian violet spray, and Tetanus toxoid. The rectal examination revealed a gravid uterus positioned cranial to the pelvic brim in the abdominal cavity; the ovaries were dorsally suspended and closer together due to the ventral traction exerted by the enlarging uterus on the broad ligament. This shows that the mare was in the early second trimester. The A-Z test revealed follicular haemorrhage on both ovaries, which confirmed early pregnancy between 40 and 120 days. Considering the mating history, the mare foals a female at 10-11 months. Postnatal and neonatal care were provided, including vaccinations with Tetanus toxoid for the dam and anti-toxin for the foal. It was concluded that rectal examination and the A-Z test could effectively diagnose early pregnancy, but rectal palpation can further diagnose advanced pregnancy, unlike the A-Z test in Sudanese horse breeds. However, the availability of an experienced sonographer limits this case management.

**Keywords:** Bruises, Diagnosis, Early pregnancy, Management, Mare

### INTRODUCTION

Gestation is the period that begins immediately following fertilisation and ends with the delivery of the foal. Generally, the gestation period for a mare ranges from 320 to 370 days, with an average of approximately 338 days (Jillian & Kari, 2024). Early pregnancy is simply known as the first trimester in mare, which is day 0 to approximately 114 days of the gestation period (Lucia *et al.*, 2025). Variations in gestation length can occur due to factors such as breed, season, sex of the foal, and the mare's body condition (Jillian & Kari, 2024).

Pregnancy diagnosis is a crucial tool for assessing reproductive management effectiveness in herds, stables, and flocks (Purohit, 2010; Khumran, 2023). The methods for diagnosing pregnancy in domestic animals can be categorised into several types: Visual Methods (History of mating, non-return to estrus, mammary gland development, and abdominal distention), Biological and Chemical Tests (Hormonal assays and milk ejection tests), Clinical Examinations (Rectal palpation, vaginal examination, and abdominal ballottement) and Imaging Techniques (X-ray and ultrasonography) (Thangamani *et al.*, 2018). Among these, clinical methods are commonly employed. For example, in

cattle, buffaloes, and mares, recto-genital palpation and transrectal ultrasonography are preferred for accurate and early pregnancy diagnosis compared to other tests (Purohit, 2010; Thangamani *et al.*, 2018; Khumran, 2023). These methods help rule out fetal death and twin pregnancies (Thangamani *et al.*, 2018). An ideal pregnancy test should deliver accurate results early in gestation, possess high sensitivity and specificity, be cost-effective, and be easy to administer (Ciplak, 2024).

In mares, sensitive diagnostic methods are essential for confirming early pregnancy (Thangamani *et al.*, 2018). While ultrasonography provides reliable results, alternative methods such as rectal palpation, biological, or chemical tests can serve as effective substitutes when imaging techniques are unavailable or if the mare's temperament is a concern. Rectal palpation is a cost-effective and straightforward method for early pregnancy diagnosis, but requires experience. Pregnancy test kits may yield false positives if the fetus dies after the formation of endometrial cups (Thangamani *et al.*, 2018). Rectal palpation, a well-established method in veterinary medicine, determines pregnancy status by palpating pregnancy-related structures (Jaskowski *et al.*, 2019). In the early stages of pregnancy (14 to 21 days post-ovulation), the cervix is tightly closed and elongated. The uterine tone increases, complicating the detection of endometrial folds by 14 to 18 days (Patricia, 2024). The developing conceptus follows a predictable growth pattern; an experienced examiner may feel the embryonic vesicle as a 3.5 cm bulge at the base of one uterine horn around 25 to 28 days of gestation. By 30 days, the uterine horns are smaller and firmer, with the conceptus recognisable as a 4 cm bulge. The conceptus reaches about 5 to 7 cm in diameter by 42 to 45 days and grows to 6 to 8 cm in diameter and 8 to 10 cm long by 48 to 50 days, beginning to involve the uterine body (Patricia, 2024). The conceptus occupies nearly the entire gravid uterine horn and half of the uterine body, measuring 8 to 10 cm in diameter and 12 to 15 cm long, while the non-gravid horn remains small but firm at day 60. After 85 days, the conceptus becomes less turgid, facilitating fetal palpation. By 90 days, it fills the entire uterus, extending into the abdominal cavity (Patricia, 2024). Between 100 and 120 days of gestation, the gravid uterus rises above the pelvic brim, causing the ovaries to move closer together due to pressure from the enlarging uterus (Patricia, 2024). After 150 days, the ovaries are typically not palpable during rectal examinations. As the gestation progresses, the growing uterus may shift lower in the abdomen, becoming difficult to reach in mid-gestation, but becomes more accessible again in late gestation.

Equine chorionic gonadotrophic (eCG), formerly known as pregnant mare serum gonadotrophin, is a hormone secreted by specialised cells in the foetal membranes during early

equine pregnancy. These cells form endometrial cups in the maternal endometrium, where eCG is produced. It can be detected in maternal blood around 37–40 days after ovulation, peaking between days 55 and 70, and typically declining and disappearing by days 100 to 140 as the endometrial cups degenerate (Canisso *et al.*, 2016; Patricia, 2024). Various factors influence eCG levels during gestation, including mare size, previous pregnancies, diet, conceptus paternity, fetal gender, twin pregnancies, endometrial folding, and uterine environment (Canisso *et al.*, 2016). False negatives may occur if samples are collected before day 35 or after day 90, while false positives can arise if embryonic death occurs after day 35, as eCG levels may remain elevated despite a non-viable foetus (Canisso *et al.*, 2016; Patricia, 2024). Since eCG presence does not confirm fetal viability, measuring estrone sulfate levels after day 100 is recommended when eCG is detected. Estrone sulphate, produced by the foetus, is a more reliable indicator of viability, with its concentrations increasing in plasma and urine after 60 and 150 days of pregnancy, respectively. These tests can effectively indicate pregnancy if breeding and ovulation dates are known (Patricia, 2024).

One historical milestone in pregnancy diagnosis is the Aschheim-Zondek (A-Z) test, developed in 1927 (Kelley, 2010). This bioassay was among the first tests to detect early pregnancy, offering 98.9% accuracy for pregnancies between 40 and 120 days of gestation in a Sudanese mare (Patricia, 2024). Although cost-effective, it was later replaced in 1960 by immunoassays, which were more practical and did not require animal sacrifice (Kelley, 2010). This method, despite its accuracy, there is a dearth of information on its use, even in situations where the personnel are not experienced and have no access to an ultrasound machine, and some may not be aware of the bioassay method. Due to the absence of an ultrasound machine, this method was employed together with rectal palpation to diagnose pregnancy at the early stage, and subsequent stages using rectal palpation only.

The uniqueness of this case report is using the Aschheim-Zondek (A-Z) test and rectal palpation in the diagnosis of pregnancy in a Sudanese mare; modification of the A-Z test by reducing the number of the sacrificed guinea pigs to two (2) from four (4); increasing the volume of serum from 0.5 ml daily for two (2) to four (4) days to 1.5 ml once, hence reducing stress to the guinea pig and the clinician; reduced the number of days from 5 days to two (2) for the guinea pig sacrifice when compared to report of (Thangamani *et al.*, 2018) and we achieved similar results. This implied the number of days to have the result is reduced from five (5) days to two (2).

Reducing the period of having results is imperative because the earlier the pregnancy diagnosis is performed, the more profitable for the horse breeders (Sertich, 1997). The

clinicians and breeders who are less experienced and do not have an ultrasound machine, especially in local settings, may employ these methods to diagnose pregnancy in a Sudanese-bred mare. This implied that the modified A-Z test can serve as an alternative method that can yield timely results at early pregnancy between 40 and 120 days of gestation while reducing the number of animals to be sacrificed.

Furthermore, "ecchymosis" is the medical term for bruises caused by damage to capillaries. When capillaries rupture, blood leaks into surrounding tissues, causing discolouration ranging from light blue or purple to dark red or black. While most bruises fade without treatment, they can sometimes indicate deeper injuries requiring medical attention (Cleveland, 2023).

This case report aimed to confirm the mare's pregnancy at an early stage, subsequent stages, and manage the sustained bruises.

## CASE PRESENTATION

### CASE HISTORY

A 4-year-old Sudanese mare weighing 300 kg was requested to be diagnosed for pregnancy. On visitation to the stable, the mare was managed intensively with 2 stallions and a foal, fed on wheat bran, grains, and grass, with a mineral block available. The mare mated with a stallion 3 to 4 months ago.

### MEDICAL HISTORY

There was a history of medication using Gentamicin, Albendazole suspension, and multivitamins, as well as vaccination using Tetanus toxoid.

### CLINICAL EXAMINATION

Physical examination revealed a distended abdomen, and the mare was healthy from the vital parameters (Temperature, pulse and respiratory rates) taken. The temperature, pulse rates and respiratory rates were 37.6 °C, 33 beats per minute, and 13 cycles per minute, respectively.

### INVESTIGATIONS

Rectal examinations and serological tests were conducted. (Figures. IA and IB). Five (5) ml of blood sample was taken from the jugular vein of the mare and placed in a plain sample bottle to harvest serum. Due to the unavailability of obstetrical gloves, an improvised pair of examination gloves was used per rectum to examine the mare for pregnancy. Rectal examination was carried out after carefully following the protocols involved. The left hand was gloved and lubricated with soap, then introduced slowly into the rectum in a cone-shaped manner, where the faeces were evacuated. The examination was paused whenever the mare strained, resuming only once she relaxed. The serological test was carried out using the Modified Aschheim-Zondek test (A-Z test), which we re-modified and applied the 3Rs of research

as follows: 5 ml of blood sample was collected from the jugular vein of the mare, and 1.5 ml of serum was harvested from it. An immature guinea pig weighing 356 g and between 3 to 5 weeks of age (Kristin, 2024) was inoculated subcutaneously with the harvested serum. Two days post-inoculation, the guinea pig was sacrificed for postmortem examination (Figures. IIA and IIB). Ten (10) days later, a second blood sample was collected from the mare, and the harvested serum was inoculated subcutaneously into another immature guinea pig weighing 351 grams. The guinea pig was sacrificed after two days (Figures. IIIA and IIIB).

Case 2: During follow-up, an emergency call was received from the client; on visitation to the stable, it was discovered that the mare had fallen into an abandoned, inactive suck away and had sustained bruises. The bruises were on the forelimbs, the thigh, and the neck region; they were not superficial and required medical attention. These injuries occurred when the owners attempted to exercise the mare and she accidentally stepped onto the abandoned suck away, leading to her fall (Figures. IVA and IVB). The body temperature, pulse rates and respiratory rates were 37.5 °C, 30 beats per minute and 14 cycles per minute, respectively, at that time.

## RESULTS

The time of the first rectal examination revealed that the gravid uterus was positioned cranial to the pelvic brim in the abdominal cavity; the ovaries were dorsally suspended and closer together due to the ventral traction exerted by the enlarging uterus on the broad ligament. During mid-gestation, the gravid uterus was difficult to reach and positioned ventrally in the abdomen on rectal palpation. In late gestation, the gravid uterus was enlarged, and its dorsal surface could be felt. The post-mortem examination of the exteriorized reproductive tracts revealed follicular hemorrhage on both ovaries (Figure V). The second blood sample taken from the mare on day 10 showed no follicular hemorrhage on either ovary (Figure VIA), but there was evidence of an enlarged uterus and uterine horns (Figure VIB). The bruises sustained by the mare were treated by disinfecting them with hydrogen peroxide and applying Bio-Blue spray (Neomycin sulphate 2g + Blue Methylene 500mg) topically. Tetanus toxoid 1ml x 1/7 i.m. (Dano Vaccines and Biologicals PVT LTD), and Diclofenac sodium injection 2.2 mg/kg x 3/7 i.m. were also administered. The bruises healed completely after two weeks. The mare subsequently foaled a female at approximately 10-11 months of pregnancy at night (Figure VI). Postpartum and neonatal care given include injection of the mare with Tetanus toxoid 1 ml x 1/7 i.m. (Dano Vaccines and Biologicals PVT LTD) and the foal with Tetanus antitoxin 1500 IU x 1/7 SC and i.m. (Jiangxi Institute of Biological Product Inc.). The foal's umbilical cord was

detached using the three-forceps technique, followed by spraying Bio-Blue spray (Neomycin Sulphate 2 g + Blue Methylene 500 mg) topically. By day two, the foal was suckling from the dam (Figure VIIA), and by day seven (7), both the foal and dam were active and healthy (Figure VIIB).



**Figure I:** Rectal examination of the pregnant mare using an improvised hand glove indicated by an arrow (A), Blood sample collection from the mare for bioassay test (B)



**Figure IV:** The abandoned suck way (A) and bruises sustained by the mare on a limb (B)



**Figure II:** The first inoculated guinea pig (A) and sacrificed 2 days post inoculation (B)



**Figure V:** The follicles haemorrhage formed on both the left and right ovary, indicated by arrows



**Figure III:** The second guinea pig inoculated after 10 days (A) and sacrificed after 2 days (B)



**Figure VI:** No follicle haemorrhage formed on both the left and right ovaries (A). Enlarged uterine body and horns indicated by arrows (B)



**Figure VII:** The mare foals a female (indicated by an arrow) at 10-11 months of pregnancy



**Figure VIII:** The foal suckling milk from the dam on day 2 (A). The foal and the dam were allactive and healthy on day 7 (B)

## DISCUSSION

The rectal palpation made in the beginning showed the mare was in the early second trimester, which agrees with the findings of Kelley (2010), who reported the presence of follicular haemorrhage on the ovaries at early gestation. These haemorrhages on the follicles can be attributed to the FSH-like activity of the Equine Chorionic Gonadotrophic (eCG) hormone present in the serum from the mare's endometrial cups, along with the immaturity of the laboratory animal, as reported by Kelley (2010), and this is an indication for pregnancy in a mare. In contrast, the second blood sample, an enlarged uterus and uterine horns taken from the mare on day 10, it revealed negative results for pregnancy due to the absence of FSH-like activity of eCG because other hormones, such as estrone sulfate produced by the embryo, were dominant, and this agrees with the findings of Kelley (2010) who reported absence of follicular

haemorrhage on the ovaries after 120 days of gestation. During mid-gestation, on palpation, the fetus is beyond reach in a mare, and this agrees with the findings of Patricia (2024), who reported that the gravid uterus might be difficult to reach because it is located ventrally in the abdomen. In the late gestation, our findings concur with the findings of Patricia (2024), who also stated that the conceptus' dorsal surface comes back into reach. The bruises, which were sustained, treated, and healed within two weeks, agree with the findings of Cleveland (2023); Carol (2024) reported that most bruises heal within two weeks and may last a month or longer. This can be due to the management instituted, leading to the absence of infection at the bruised site, facilitating the healing process. The postpartum and neonatal care given is to prevent the mare from being infected by Tetanus and neutralising possible toxins that may enter the umbilicus because the foetus was delivered on a soil surface where *Clostridium tetani* may be present. The foal's umbilical cord was detached and healed due to the absence of infection. The dam and the foal were active and healthy, evident from their activities on Figure VIII. It was concluded that rectal examination can be performed at all stages of pregnancy and serves as a confirmatory method. In contrast, the A-Z test is applicable during early gestation and is considered reliable; it can be used where immunoassay and ultrasound machines are unavailable. The A-Z test is also suitable for use when a professional radiographer is not available or when clients cannot afford immunoassay or ultrasound services and do not require immediate results. Despite its advantages, the A-Z test has several limitations, including a longer processing time, an inability to determine foetal viability, the requirement for animal sacrifice, and relatively higher costs compared to rectal palpation. Horse owners should always isolate pregnant mares in safe environments and minimise stress to prevent potential losses in their stables.

**Conflict of Interest:** There is no competing interest concerning this publication.

**Ethical Guidelines:** There is no ethical committee, but the number of guinea pigs sacrificed was reduced to two and stress to the guinea pigs was minimised.

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