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Ultrasonographic features of the mule embryo, fetus and fetal-placental unit

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Abstract

The aim of this study was to establish baseline ultrasound data concerning the mule conceptus during gestation. Ten multiparous Trotter mares were artificially inseminated with chilled semen from an Amiatino jack donkey. Daily transrectal ultrasonography was carried out from the day of ovulation until Day 50 of gestation to determine the following: first detection of the embryonic vesicle (EV), mobility phase, EV diameter, day of EV fixation, changes in EV shape, date of yolk sac regression and embryo crown-rump length. Monthly ultrasonic assessments from Day 50 of gestation to term were carried out. These assessments included an evaluation of fetal well-being and the growth of the mule conceptus, which were monitored using the following variables: cardiac activity, fetal activity and presentation, fetal fluid echogenicity, combined thickness of the utero-placenta unit and fetal orbital and aortic diameter. Mule EV first detection was observed earlier (37% at Day 8) than that observed in the equine pregnancy. EV diameter at first detection was 4.6 ± 1.1 mm. At Day 10, 75% of EVs were detected. EV fixation occurred on Day 17.1 ± 1.1, with a mean EV diameter of 2.5 ± 0.2 cm. EV growth rate was 4.04 mm/day from Days 11 to 16, 0.4 mm/day from Days 16 to 28 and 1.78 mm/day from Days 28 to 45 of pregnancy. The embryo proper was first detected on Day 19.9 ± 1.9 (average length 2.4 ± 1.4 mm), and the embryonic heartbeat was first detected on Day 24 ± 2.4. The fetal carotid pulse was observed at six months of gestation and provided a good means by which to estimate fetal cardiac activity in advanced gestation. The fetal heart rate was recorded from Month 2 of gestation to term. The mean ± SD of the combined uteroplacental thickness was assessed at the cervical-placental junction and at the ventral abdomen in mares between Months 2 and 5 until term, respectively. An abnormal fetal-placental unit and fetal inactivity was observed in association with abortion. Mule-conceptus biometric measurements correlated significantly with the gestational age, and these data were used to predict an unusually large mule fetus, which might result in dystocia. In conclusion, we can assume that early diagnosis of pregnancy failure and assessment of fetal biophysical profile and growth charts could improve the chances of gestation completion in mule-pregnant mares. The early detection of mares at risk for an abnormal pregnancy or delivery may increase the success of prompt treatments, therefore preventing costly emergency procedures and allowing proper obstetrical and neonatal assistance.

Keywords: Mule; Pregnancy; Embryo; Fetal biophysical profile; Ultrasonography

1. Introduction

The different species of the genus Equus can interbreed and usually produce hybrid infertile offspring [1,2]. There are well-documented reports of fertile mules giving birth to viable offspring [3–5]. The mule,

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derived from the mating of a horse mare to a jack donkey, has been propagated since ancient times due to its usefulness in human activities. Recently, in Italy, the mule has become more and more interesting for tracking, and it is suitable for the exploitation of mountain resources as well as a valuable tool for psychomotor rehabilitation programs.

There are reports of pregnancies and live foals in donkeys as well as the transfer of horse embryos to cycling mule recipients [6,7]. Furthermore, three mule foals produced by nuclear transfer of fetal somatic cells were the first cloned equids to be born in 2003 [8]. Transfer of donkey embryos to horse mares provides a useful model for early fetal death, as endometrial cups, which are a characteristic of mare pregnancy, do not develop in extra specific gestation, and abortion is generally observed in conjunction with an intense accumulation of leukocytes in the endometrium [9–11]. Failure of endometrial cup formation in donkey embryos transferred to mares is associated with a high rate (79%) of fetal mortality between Days 80 and 100 of pregnancy [10,12].

Several new reproductive techniques, such as ultrasonography, have been developed in the equine industry, thereby revolutionizing both the diagnosis and monitoring of mare biologic and pathologic reproductive events [13]. Ultrasound assessment of equine and donkey embryos and fetal-placental well-being has been found to be a valid diagnostic tool [14–20]. Abnormal fetal-placental units and equine fetal growth patterns are associated with abortion [21,22], perinatal death [23] and abnormal delivery [24]. Foal weight is correlated positively with both allantochorionic extension and weight. Furthermore, placental abnormalities are highly associated with fetal demise or distress, even if they seem relatively minor or are not evident at first [25]. Equine fetal growth depends on adequate placental development; thus, biometric measurements are useful indicators of unfavorable intrauterine conditions [26]. Fetal growth parameters have been reported in Thoroughbred and Standardbred horses just prior to parturition [22].

In accordance with the trend set in equine perinatology, methods for assessing equine interspecies fetal-placental well-being using both transabdominal and transrectal diagnostic sonography have been developed for mule pregnancies by Paolucci et al [27]. The purpose of these techniques is to identify mares at risk of an abnormal pregnancy or delivery, thereby allowing closer supervision during the peripartum period and earlier detection, treatment and, possibly, prevention of costly neonatal disease. Perinatal death still accounts for a large percentage of mule-foal mortality, despite extensive improvement in neonatal intensive care medicine. Therefore, the objectives of the present study were to further validate the ultrasound assessment of equine fetal-placental well-being by using it throughout pregnancy and to establish reference guidelines for different gestational stages for an interspecies pregnancy, as the main reports have generally focused on equine gestation.

2. Materials and methods

2.1. Animals and treatments

The study was carried out at the Teaching Farm of the University of Perugia, School of Veterinary Medicine (43° 001614 N, 12° 367062 E) during breeding seasons from 2005 to 2009. A total of ten healthy 15 ± 7 years old and 500 ± 50 kg body weight multiparous Trotter mares withdrawn from the studbook were assigned to the experiment; by this way a total of 28 pregnancies were monitored in a 5 y period. All animals were maintained free in a paddock, and nutritional requirements were provided using a diet based on hay *ad libitum* and pelleted concentrate throughout the pregnancy. The mares underwent a breeding soundness examination to detect the presence of the abnormalities of the genitalia [28,29].

Early in February, teased mares that showed estrous behavior underwent daily transrectal ultrasound examinations of their genital tract to evaluate ovarian follicle development. Once a 35–45 mm or greater follicle was identified, animals were artificially inseminated every other day with 15–30 mL of chilled semen diluted in Kenney glucose-skim milk extender to 40×10^6 spermatozoa/mL until ovulation was confirmed (ovulation = Day 0). An insemination dose of at least 600×10^6 progressive motile spermatozoa was used. Semen derived from the Amiatino jack donkey was shipped from a stud farm located in the northeastern region of Italy and was used within 24 h of collection.

The embryonic and fetal parameters included in this study were selected to assess the well-being of the mare, the well-being of the fetus, the integrity of fetal membranes and the neonate's health.

2.2. Age effect on breeding efficiency

In order to evaluate age effect on breeding efficiency, the mares were assigned to the three different age Groups: Group I with an interval of 5–10 y, Group II 11 to 15 and Group III more than 15 y. Mean number