



Clinical, ultrasonographic, and endocrinological studies on donkey pregnancy

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ABSTRACT

Although donkey breeding has gained new interest in the past two decades, knowledge about donkey reproduction is still scarce, particularly on jenny pregnancy. The aim of this study was to describe the ultrasonographic and endocrine profiles of the physiological pregnancy in the jenny. The study was performed on 12 pregnancies of 7 Amiata donkeys from Day 10 after ovulation to delivery. Because three pregnancies, respectively at weeks 42, 44, and 45, were considered pathologic and treated pharmacologically, data collected from 2 weeks before diagnosis to the end of pregnancy were removed from the analysis. Average length of the normal pregnancies was 353.4 ± 13.0 days (range, 339–370 days). Timing, dimensions, and development during the first phases of embryonic growth, evaluated using transrectal ultrasound, were similar to that previously described in jennies and mares: first detection of embryonic vesicle was at 11.8 ± 1.3 days of gestation and diameter was 6.5 ± 1.9 mm, loss of spherical shape occurred at 18.5 ± 1.4 days, and embryo and heart beat were first seen at 22.0 ± 1.1 and 25 ± 1.1 days, respectively. The intrauterine growth in the second half of pregnancy, evaluated using the transrectal and transabdominal approach, also showed strong positive correlations, similar to that reported for the mare. The trends of the combined thickness of the utero-placental unit and the echogenicity of the amniotic and allantoic fluids are examples. The diameters (mm) of fetal chest, eye orbit, and aorta increased throughout pregnancy and were 40.6 ± 2.9 , 8.7 ± 1.5 , and 3.5 ± 0.7 , respectively, at week 13, and 190.9 ± 12.0 , 21.4 ± 1.5 , and 30.6 ± 1.8 at the last evaluation before parturition. In contrast, heart rate decreased as pregnancy progressed. Regression analyses between these parameters and day of gestation were statistically significant ($P < 0.001$). All fetuses consistently showed some intrauterine activity. Maternal plasma progestagens and estrone sulfate concentrations followed a pattern similar to that seen in mares, although the prepartal progestagen peak was lower in jennies. This study provides a range of ultrasonographic and endocrine values for normal pregnancy in jennies.

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1. Introduction

The world donkey population is estimated to be 44 million [1]. They are located primarily in the regions of the

world where the infrastructure is not well developed, where they are used as working animals. In contrast, donkey numbers are limited in the more industrialized countries, where autochthonous breeds are at risk of extinction. In Italy, a 96% decrease in the donkey population occurred between 1939 and 1996, but in the past two decades the interest in donkey breeding has increased because, among other reasons (e.g., working or companion

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animals, pet-therapy) they have been used as milk-producing animals [1,2]. Thus, previously donkey reproduction was not much studied, but knowledge in this field has now increased in importance.

The use of ultrasound to evaluate pregnancy and fetal wellbeing has been used in the horse since 1980 [3]. In donkeys, a limited number of studies evaluated the development of the equine conceptus up to Day 60 of pregnancy [4–7]. There are no data, however, beyond this stage of pregnancy, with the exception of a study on fetal sex determination [8]. In the horse, however, clinical use of transabdominal ultrasound (TAU) in later pregnancy established normal values for several fetal and maternal parameters, such as fetal size, growth, and activity [9–13], and mother-fetus exchanges [10,11,13,14]. Recently, a study described the ultrasonographic features of the mule (hybrid derived from a horse mare and a donkey jack) embryo, fetus, and fetal-placental unit throughout pregnancy [15]. This type of examination has clinical application because it enables the diagnosis of some abnormalities before parturition [9,10,16–18].

Endocrinology during gestation has been studied in depth in the mare [19–33]. In contrast, data for the donkey are scarce, mainly limited to the first half of pregnancy [19,20] or to its final phases [34,35], except for one study which describes the progesterone and estradiol profiles throughout the entire jenny pregnancy [36]. In mares, the two hormones most frequently evaluated during pregnancy are progesterone/progestagens and estrogens. Progesterone is produced initially by the primary corpus luteum and later by the accessory corpora lutea; its role in maintaining the pregnancy is progressively taken by the progestagens of fetoplacental origin, which are significantly elevated in the last weeks before parturition and decrease in the 24 to 48 hours before parturition [21,22]. Progesterone is at basal levels (<1 ng/mL) from half of gestation on, and other progestagens might exceed 500 ng/mL in late gestation [23] and act on mammary gland development and electrolyte changes in milk [24]. All progestagens originate from pregnenolone, produced in large amounts by the fetus, released into the umbilical artery and converted by the uteroplacental unit into different progestagens, some of which return to the fetus for further metabolism and some are excreted directly into the maternal circulation [25,26]. Many clinical condition of the placenta or fetus might alter progestagen metabolism and hence plasma concentrations [24,27–29]. Estrogens are present in high concentrations in the midphases of equine pregnancy (up to 1000 ng/mL) and they reflect the huge development and regression of the fetal gonads [22,30,31]. The main equine estrogens are estrone sulfate, estradiol-17 β , equilin, equilenin, which originate from dihydroepiandrosterone and related compounds [32]. Their main function is to stimulate blood flow in the placenta and endometrium, and the synthesis and storage of PGF_{2 α} in the myometrium [22–26]. Because the precursor originates from the fetal gonads, measurement of plasma concentration of estrone sulfate, which is the most abundant estrogen in equine pregnancy, might provide an estimate of fetal viability and wellbeing [27,29,33]. Overall, the previous studies on endocrinology of pregnancy in donkeys showed

similarities to that found in mares, although some differences (e.g., in serum eCG concentrations, or prepartal progestagen profile) were observed [19,20,35,36].

The aim of this study was to describe the physiological aspects of ultrasonographic findings and endocrinology during pregnancy in jennies.

2. Materials and methods

2.1. Animals

The study was conducted between 2006 and 2009 on 12 pregnancies of seven jennies belonging to the Amiata donkey breed. Jennies were 5 to 12 years old, weighed 300 to 350 kg, and were kept in collective paddocks at the Veterinary Teaching Hospital, Department of Veterinary Sciences, Pisa University (43° 43' N), fed with a balanced ration of meadow hay *ad libitum* and commercial equine feed. Close to parturition, jennies were kept in stables at night. All jennies were treated for gastrointestinal parasites and vaccinated for influenza and Equine herpes virus (EHV) 1 and 4 using the American Association Equine Practitioners guidelines for horses.

2.2. Management of estrous cycle and parturition

Jennies in estrous were examined daily using ultrasound and either mated or inseminated every second day until ovulation was confirmed (day of ovulation = Day 0). At parturition, presentation and viability of the foals was evaluated, and sex of the foals was registered.

2.3. Ultrasonographic assessment from pregnancy diagnosis to Day 145 (week 21) = first phase

On eight pregnancies from seven jennies, transrectal ultrasonography (Toshiba JustVision 200, semiconvex probe PVF-738F, 5–7 MHz) was performed every second day between Days 10 and 40 and weekly between Days 40 and 145 (week 21) after ovulation. Day 40 was considered the limit between embryonic and fetal phase [21]. The parameters evaluated on the embryonic vesicle (EV) were the following: appearance and diameter of the EV, day and size of the fixation of the EV, day of the loss of spherical appearance of the EV, first appearance and size of the embryo, onset of heart beat, first appearance of the allantoic sac, and of the umbilical cord. In the fetus, the crown-rump length, the first appearance and size of the thorax, eye orbit and aorta, and the first appearance of the stomach were recorded.

2.4. Ultrasonographic assessment from Day 150 (week 22) to term = second phase

Seven jennies, with a total of 12 pregnancies, were monitored weekly from Day 150 to foaling using transrectal ultrasound (TRU; Toshiba SSA-350A Corevision Pro, Toshiba semiconvex probe PVF-738F, 5–7 MHz) or TAU (Toshiba SSA-350A Corevision Pro, Toshiba convex probe PVF-375AT, 3 MHz). The following parameters were studied: combined thickness of the uteroplacental unit at the cervical pole

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