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Review Article

A Review of Reproductive Biology and Biotechnologies in Donkeys

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ABSTRACT

Donkeys have been valuable to people in different cultures and countries for centuries. Nowadays, they are used in recreation, in therapeutic activities, and mainly for the production of mules. Donkeys and horses show reproductive similarities and differences. The reproductive cycle and gestation length in jennies are longer than in mares, and they exhibit some special features in their reproductive behavior. Seasonality is less evident, with other factors besides photoperiod influencing it. Jacks' reproductive organs are larger than those of stallions, and they also take a longer time to achieve ejaculation. Insemination with raw semen is carried out on many farms, and some efficient protocols with cooled semen have been applied; however, use of frozen-thawed semen remains a challenge. Embryo transfer results have improved over the last years, but to become an effective reproductive technique, pregnancy rates need to be increased. Therefore, a deeper knowledge of reproductive physiology would help to achieve suitable protocols for applying reproductive biotechnologies in donkeys.

1. Introduction

Donkeys belong to the perissodactyla order, equidae family, including the *Equus* genus, with five subgenus: wild horse (*Equus caballus* or *Equus przewalski*), wild donkey (*Equus hemonius*), donkey (*Equus asinus*), zebra (*Hippotigris*) and Grevy zebra (*Equus grevyi*).

Domestic donkeys usually weigh between 80 and 480 kg and range between 80 and 160 cm in height. On average, they live 30–35 years and can still be actively working even at an advanced age.

Donkeys are valued for their easy care, their resistance to diseases, and their physical endurance [1].

In the old days, donkeys had many more diverse uses than they do today. They were an important workforce in agriculture, commerce, and militia, as well as a source of milk, hide, and meat.

Currently, the use of this species has diminished. In Central Europe, donkeys are seen mainly as a recreational animal and on occasion are used in programs of therapy with animals. However, an increase in the demand for donkey milk and its by-products has been observed together with that of meat products that are commercialized in the gourmet markets and as specific dietary products [1].

To obtain a more robust animal, the breeding of mules became widespread. Achieving a good mule biotype depends largely on the adequate selection of the male donkey and thus reproduction of large breeds was initiated. This led to their use by the militia (Fig. 1) and for tourism and transport in mountain areas, all of which are important in Argentina (Fig. 2).

However, not every donkey will be interested in breeding mares. Jacks usually need to have grown up with mares to show libido when in their presence. Hence, reproductive biotechnologies may help to solve the problems related to this issue, in addition to other known advantages such as a more efficient use of semen and genetic improvement. The conservation of endangered breeds is another very important advantage to applying reproductive biotechnology as for example, just in Europe, the population decline is of up to 80% in the 20th century [2], and the number of donkeys in China have decreased dramatically from 9.56 to 5.42 million heads between 1998 and 2015 [3]. Thus, artificial insemination, sperm cryopreservation, embryo transfer (ET), and newer technologies need more studies to deepen our current knowledge of reproductive physiology to widen the prospective application of assisted reproductive techniques.

2. Female Reproduction

The jenny is similar to the mare in several reproductive aspects. They reach puberty at ages 1 or 2, although showing less seasonality

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Fig. 1. Mule being handled and trained to the saddle by the military in Argentina.

than mares [4,5]. A longer estrus cycle is reported, with a range of 20–40 days (average 24 ± 1 day vs. 21 ± 1 day in the mare). Estrus may last 6–9 days, with ovulation occurring in the last 24 hours. Dominance is established when follicles are about 25 mm in diameter [6]. Depending on the reproductive stage, up to 13 follicles of different sizes can be observed per ovary. Follicular growth averages 2.7 mm per day and ovulatory follicle diameter may vary according to the breed: 44 mm in Catalonian [7], 30–33 mm for Poitou, 35–40 mm in Pega and Mammoth, and 32 mm for North African jennies [6]. A slightly higher frequency of ovulation in the left ovary than the right has been observed in several studies [7–10]. Lemma et al [11] observed that about 80.5% of large ovulatory follicles (>20 mm) changed their shape from spherical to oval, irregular, or ellipsoid before ovulation. The remaining 19.5% continued with a spherical shape until ovulation. Double ovulation rate ranges from 5% to 32%, yet twins are rare [6]. Endometrial edema may be observed approximately 1 week after the disappearance of the corpus luteum (CL) and increases towards the day of ovulation. A highly significant positive correlation was found between the cross-sectional diameter of the uterus and the size of the dominant follicle, and a similar positive correlation was observed between the score of the endometrial folds (scale 0–3) and the size of the dominant follicle. However, the score of endometrial folds was not a reliable predictor of imminent ovulation, as has been reported in mares [11]. The cervical appearance changes with the stage of the estrus cycle, relaxing during estrus, at which time it is accompanied by an increase in vaginal mucous secretions [12]. The interovulatory period is longer than in mares, 24.9 ± 0.7 days for standard jennies and 23.3 ± 2.6 days for mammoth jennies (vs. 21 ± 1 day in the mare). The average life span of the CL ranges between 15 and 20 days (19.3 ± 0.6 in standard jennies and 17.4 ± 2.6 days in mammoth jennies) [13,14]. Sometimes, CLs may exhibit a homogeneous echotexture the first day after ovulation and in other cases, jennies show a CL with a nonechogenic central area [7].

Fig. 2. Mules bred for use by the military, transport of cargo, and for touristic activities in the South American Andes.

3. Seasonality

Seasonality is a controversial subject and is likely influenced by photoperiod combined with other factors such as breed, nutrition, health, and environmental conditions [6].

Ovarian activity, pregnancy, and parturition appear to be much less seasonal in domestic donkeys than in wild asses [15,16], despite the report that melatonin concentrations in jennies and mules are considerably higher (90 pg/mL^{-1}) than in mares (24 pg/mL^{-1}), suggesting that melatonin concentrations are genetically determined [17].

Some differences are reported, according to the place of study: in southern USA, jennies may cycle all year around, while in Wisconsin (latitude 43° N) seasonality is less evident than in mares (64% of jennies are cycling in December, whereas under 35% mares cycle in the same month). In Brazil (latitude 19°51′ 06″S), a marked seasonality has been observed (54% of the females present anestrus) and in Morocco, field observations show that the peak-birthing season is March–May [6].

In Spanish breeds, Galisteo and Perez-Marin [18] reported interovulatory intervals in jennies decreasing from November to May (from 24.6 to 17.6 days), whereas in a study carried out in Martina Franca jennies, the length of estrous cycles was significantly longer in summer than in autumn and winter, and diestrus was significantly reduced during summer compared with the other seasons [19]. In Ethiopian jennies, the interovulatory interval was shorter during the season of higher sexual activity than during the season of lower activity, corresponding to short rainy and dry seasons, respectively. The authors attributed this to a different influence from the classical photoperiod effect observed in the temperate regions. They consider that owing to forage availability being affected by seasonal rain, nutritional status is the most likely factor affecting ovarian follicular activities [11].

4. Gestation and Parturition

Gestation length ranges between 333 and 395 days, with an average of 371 days [6,20] or 363 days for Spanish breeds [18]. Pregnancy length seems to be affected by foal gender, with cases of longer pregnancies in jennies bearing male fetuses (376 days) compared to pregnancies bearing females fetuses (371 days) [20]. Other aspects, such as the year of foaling, the month of ovulation and parturition, the birthweight of the foal, and the age of the jenny, did not significantly influence pregnancy length in Martina Franca jennies [20], although in Spanish breeds, the date of ovulation and of parturition did seem to affect pregnancy length in a similar way to that observed in mares, with longer pregnancies ensuing when ovulation was at the beginning of the season. Thus, duration of gestation became shorter as the breeding season advanced [5,18].

Jennies have a diffuse epitheliochorial placenta, as do mares, weighing a 12% of the neonate's birthweight [21]. However, despite microcotiledons being more complex than those of mares (showing a greater degree of ramifications in the jenny), they are less efficient in terms of kg of foal birthweight per square meter of feto-maternal contact [22].

Combined thickness of uterus and placenta (CUPT) has been measured in Martina Franca jennies and showed a significant increase after the 6 month of gestation, but this difference was significantly higher as from the ninth month of pregnancy. The