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Some reproductive characteristics in common donkey male (*Equus asinus*)- A mini review

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ABSTRACT

In contrary to most domestic livestock species, the common donkey (*Equus asinus*) is widely known as an animal with marked seasonality in reproductive activity. The annual cycle of daily photoperiod has been identified as the determining factor in sexual activity. A synthesis of the particularities of donkey reproduction is important and constitutes a basis of scientific reflection for managing asine livestock farms and establishing a well conservation plan for the different breeds around the world. It is necessary to have a perfect knowledge of the seasonal physiological changes in order to optimize the reproductive characteristics of donkeys. The objective of this paper is to review the current state of knowledge on the reproduction seasonality of common donkeys. We start with a remainder of some anatomical of the genital apparatus and sexual behavior aspects. Moreover, the investigations undertaken by many authors reveal the influence of the season on testicular biometry, histology, seminal and hormonal parameters in male donkeys populations. In conclusion, despite scientific controversy on the reproductive seasonal character in donkeys, it could likely be influenced by several factors mainly the photoperiod.

Keywords: *Sexual Behavior, Sperm, Hormone, Seasonality, Male, Donkeys*

INTRODUCTION

For as long as donkeys were known, the female was always more scientifically interested than the male. Many of the earliest accounts of donkeys were only interested in female. However, the male donkey (ass, jackass) has many interesting characteristics and there is abundant literature dating back to the past century. In recent years, donkeys have become more widely used in Central European countries by equine breeders for leisure activities in ecotourism and hippotherapy. Field reports revealed a continuing decline in world populations of the asinine species (Kugler et al., 2008) with the advent of motorization in transport and work (Figure1), this can be a risk of extinction of the species and cause a negative impact on animal biodiversity (Vlaeva et al., 2017). A synthesis of the particularities of donkey reproduction is important and constitutes a basis of scientific reflection for managing asine livestock farms and establishing a well conservation plan for the different breeds around the world (Rodrigues et al., 2021). The aim of the article is to give information about the anatomical and physiological aspects of male donkey reproduction.

Histoanatomy of the genital apparatus

The male reproductive tract consists of a pair of testes, epididymis, vas deferens, ejaculatory duct, and accessory sex glands (prostate… *etc*.).

Figure 1. Use of the common donkey (*Equus asinus*) in transport (a) and agriculture (b) before the advent of motorization

Genital tract

The donkey penis and prepuce are anatomically like stallions (Hagstrom, 2004). In donkeys, the penis in resting measures 50 cm long, with a diameter of 2.5-5 cm, and the presence of two nipples on each side of the sheath (Canisso et al., 2019). The penis body is clearly protruding along the entire length of the copulatory organ and is enveloped almost up to its tip by the bulbous spongy muscle (Chabchoub et al., 2007). The dilatation of the glans is more important in donkeys (Tibary et al., 2006) and its length increase clearly in erection, its size could range from 35.5 to 45.5 cm in diameter for a small donkey weighing 100 kg (Purdy, 2010).

The prostate forms a large glandular structure which consisted of right and left lobes, extended over the dorsal and lateral faces of the urethra (Morel, 2003). It shows an isthmus almost a centimeter thick and 2 to 3 cm long in the cranio-caudal direction, of a slate color and becoming yellowish after castration in donkeys. The prostate of the donkey is enveloped by a thick capsule which has outer fibrous and inner fibro-muscular layers. It seems that the prostate gland of a donkey is more active during the spring season compared to the other seasons of the year; this activity could be strongly related to the breeding season (Abou-Elhamd et al., 2013).

Testis and epididymis

Donkeys have larger, more globular testicles compared to stallions with smaller, laterally constricted, ovoid-shaped testes (Figure 2).

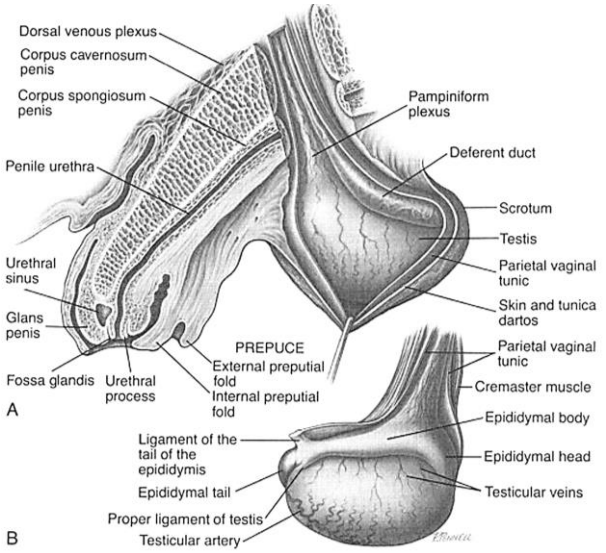

Figure 2. Chart illustrating a longitudinal section of the distal penis (A) and lateral aspect of the testis (B) in the common donkey (*Equus asinus*) (Nickel et al., 1979).

It measures on average 6 to 7 cm in wide, 9 to 10.5 cm in length and 3.7 to 5.2 cm in height (Table 1). Testicular size increases with age, nevertheless, the degeneration can be noted from the age of 15 years (Chabchoub et al., 2007). Donkeys' testicle is almost horizontal, with their long axis lightly oblique in the ventro-caudal direction without a difference between the right and left gonad (Barone, 1990). The testis of the donkey was completely descended into the scrotum around birth. It was covered by a thick tunica albuginea consisting of outer and inner fibrous layers in addition to the middle vascular one (Banks, 1993). Discrete bundles of smooth muscle cells were demonstrated in the outer fibrous layer of the tunica albuginea. These cells give the tunica albuginea a contractile function, which may aid in sperm transport (Abd-Elhafeez et al., 2017).

The epididymis is 12 to 13 cm long in the donkey and the duct can reach 70 to 85 cm. It weighs 17 to 25 g and its head receives from 12 to 23 efferent cones (Barone, 1990; Aissanou and Ayad, 2020). The corpus is relatively thick. The cauda epididymis is bulging, medially deviated and more prominent in donkeys with an average diameter of 2.45±0.08 cm (Aissanou and Ayad, 2020). The ligament of the tail of the epididymis and the testis are thick and

distinct (Chabchoub et al., 2007). The histomorphological study of the testicular compartments showed that the seminiferous tubule diameter was 222±6 µm (Neves et al., 2002); 221.51±2.32 µm (Aissanou and Ayad, 2022) and 205.6±6.65 µm (Moustafa et al., 2015). Similarly, germ cell epithelial height values in mature individuals were recorded; 68.71±1.1 µm in the Algerian donkeys (Aissanou and Ayad, 2022); 72.7±1.98 µm in Egypt (Moustafa et al., 2015) and 70.0±5.3 µm in the wild donkey (*Equus asinus africanus*) (Nipken and Wrobel, 1997).

Sexual behavior

The pre-copulatory behavior manifested by some signs such as naso-nasal contact; flehmen; nibbling and/or sniffing of the head, neck, back of the knee, flank, perineum and tail; and olfactory examination of urine or excreta without an erection or immediate copulation (Henry et al., 1998; McDonnell, 1998).

The role of vocalization appears to be an important factor in successful communication between the two partners in mating, where 78% of female vocalizations under pasture breeding management were in estrus (Henry et al., 1991). The male donkey is territorial compared to horses which keep a group of mares and it mates with jennies that approach him or cross his territory (Figure 3).

Henry et al. (1991) reported that the first mating of males was recorded during the day on pasture after 39.9±30.4 and 25.9±17.8 min of intermittent teasing periods. While the inter-copulatory interval was 88.4±71.5 and 93.3±54.5 min, when erection is achieved, the jacks return to the jennies to mate and proceed straight to the mounts once the males smelled the perineal area. For the jacks, the time from the approach in erection to ejaculation ranged 32±20 and 51±50 s. On the other hand, the time of mount to ejaculation was included 19±5.5 and 19±5.0 s. The ejaculation usually takes place after 4–8 pelvic thrusts lasting 19-30 s (Henry et al., 1991; Gastal et al., 1996; Quartuccio et al., 2011). It is also noted that from the first contact with the female (appetitive sexual behavior) to the ejaculation (consummatory sexual behaviour) ranges from 6 to 32 min (Clayton et al., 1981; Henry et al., 1991; Gastal et al., 1996). Other observations were recorded that jacks would relax on the jenny's back for five to ten seconds following ejaculation before assuming the quadrupedal station, with pelvic flares ranging from 5 to 6 before ejaculation (Henry et al., 1998). In the post-copulatory phase which takes about 15–30 min, the jack shows a total disinterest in the female, stays in the resting area, and eventually refuses the female approach (Henry et al., 1991; Quartuccio et al., 2011). The variation of erection latency observed by Costa (1991) and Veronesi et al. (2008) (1 to 45 min and 14 to 39.3 min, respectively) may well in part be due to the particularly sensitive nature of jacks to environmental disturbances such as noise, weather, and general management (Henry et al., 1991)

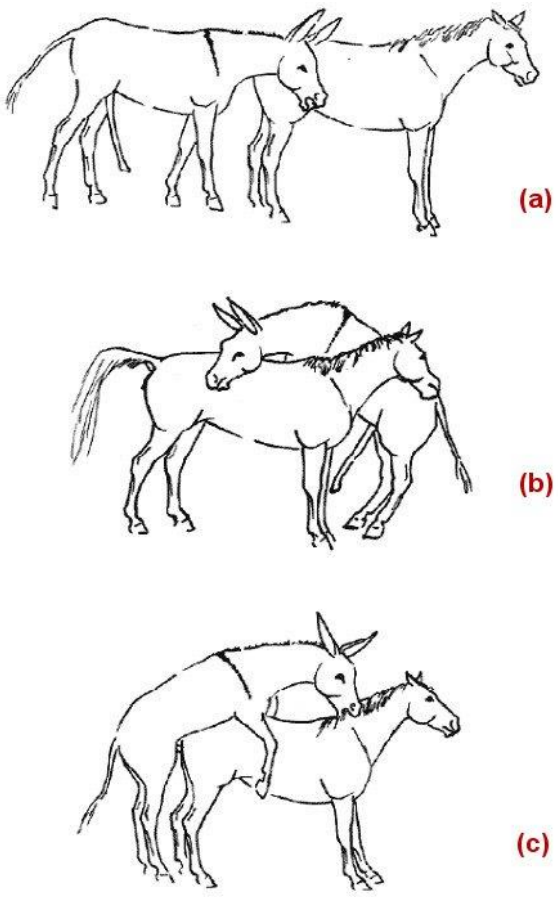

Figure 3. Donkey jack sexual behavior in presence of the female in deep estrus. (a) The donkey, with a full erection, approaches the female that is presenting typical passive behavior (top); (b) the donkey male may mount laterally the female; (c) successful copulation.

Seasonality

There are general factors that can influence the seasonal reproduction of animals, such as related to genotype and individual; and other are related to the animal's environment (Bronson, 1989). It is known that mammals generally use the duration of daylight to regulate not only their reproduction but also many other seasonal processes such as hibernation, growth, moulting, *etc*. In the literature, the scientific opinion is controversial on the seasonality character of the asine species, it likely

could be influenced by photoperiod and other factors such as breed and environmental conditions (Tibary et al., 2006). The investigations were undertaken to study the influence of the season on testicular biometry, histology, seminal and hormonal parameters are very limited in male donkey populations (Aissanou and Ayad 2020; 2022).

Photoperiod is the main environmental factor that influences seasonal patterns. Long or short days inhibit or stimulate sexual activity in animals. The light detected by the retina is translated into a neuroendocrine message by the epiphysis through the secretion of melatonin which influences seasonal reproductive activity (Reiteret al., 2018). Low or high concentrations of melatonin during daylight and nighttime respectively, are molecular indicators of photoperiod (Carcangiu, 2013).

Also, the seasonal changes in reproduction including especially hormonal activity, gametogenesis, and testicular size may show fluctuations depending on the period of the year. It is well known FSH acts specifically on Sertoli cells, playing an important role in the maintenance of qualitatively and quantitatively normal spermatogenesis. LH acts on Leydig cells to stimulate testosterone secretion (Dutta et al., 2019). Moreover, testosterone secretion, which is the major testicular androgen, is a necessary prerequisite for the maintenance of established spermatogenesis in the adult testes (Roberts and Chauvin, 2019).

The gel free volume and mass motility score in autumn were more ($P<0.05$) than in summer and winter. The pH of semen in winter and summer differed from the autumn season. The sperm concentration during summer was more than in autumn and winter ($P<0.05$). However, the live sperm percentage did not differ significantly due to season (Roy et al., 2003). Also, other observations have been reported on the biochemical characteristics of semen, which the means concentrations of glucose, cholesterol, acid phosphatase and aspartate aminotransferase activities in the seminal plasma were significantly higher in autumn and winter compared to summer (Roy et al., 2004).

In Brazilian donkeys, the seasonal effect was observed only on seminal pH among all the sperm parameters (Gastal et al., 1997). Whereas Contri et al. (2010) noted a significant difference in the volume and viability in fresh Martina Franca donkey sperm characteristics, which values were higher during the short-day season, *i.e.* in November and December. On the other hand, no significant differences in testicular morphometric traits or in seminal parameters were observed in the Italian Martina Franca donkey during the year (Carllucio et al., 2013). It was also demonstrated that the seasonal change of plasma testosterone level in the breeding season (Mar-Sep, 2.18±0.27 ng/mL) were significantly higher than in the non-breeding season (Oct-Feb, 1.50±0.18 ng/mL) in Chinese donkeys and correlated with photoperiod and temperature (Jiaha, 1983). Moreover, the seasonal variation of 17β-Estradiol differs by season because the average level in April-September was higher than in October-March.

Shuler et al. (2019) evaluated the testicular endocrine function of male donkeys (*Equus asinus*) under field conditions in Germany. A highly significant influence of season on the evolution of steroids concentrations namely estrone, estrone sulfate and testosterone through the year was recorded (Figure 4).

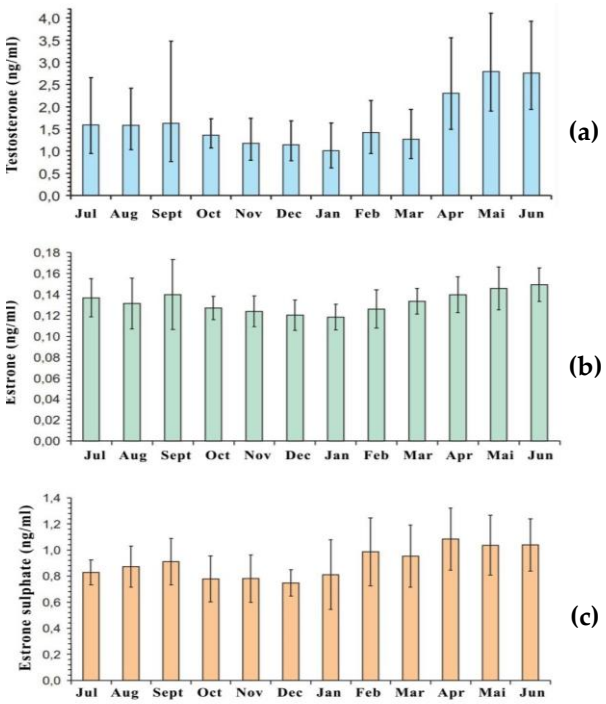

Figure 4. Testosterone (a), estrone (b) and estrone sulfate (c) secretory profile (mean ± SE) in donkeys (*Equus asinus*) in blood serum during different months of the year (Schuler et al., 2020).

The concentration values were low from November to January and high in April, May and June. Thus, the results showed that breed also had an effect on the expression of seasonality between dwarf and standard donkeys. In addition, seasonal interactions and geographical location seem to have

a considerable influence on reproductive seasonality in donkey populations around the world (Tibary et al., 2006).

Recently, it has been reported in testis histomorphological investigation that sexual activity usually occurs in winter and autumn in local donkeys from northern Algeria (Aissanou and ayad, 2022). Likewise, the highest gonado-somatic index and scrotal circumference values were recorded in the autumn and winter season. As well as the values of the testicular and epididymal biometrics were significantly higher in the autumn and winter season than in the spring and summer seasons (Aissanou and Ayad, 2020).

Table 1. Mean values (± SD) of the donkey (*Equus asinus*) breeds testis biometry.

Measurement	Algerian donkey[1]	Ethiopian donkey[2]	Martina Franca donkeys[3]	Miranda donkey[4]
Testicular length (cm)	6.8±0.2	13.4±3.78	9.6	8.04±1.2
Testicular Width (cm)	4.92±0.13	6.96 ± 1.65	6.8	6.06±1.19
Testicular Height (cm)	3.79±0.12	ND	5.2	4.63±0.86
Testicular Weight (g)	80.91±5.8	277±32.7	ND	ND
Testicular Volume (cm^3)	73.42±5.53	ND	ND	126.28±56.52

[1]Aissanou and Ayad, 2020; [2]Lemma and Deressa, 2009; [3]Carluccio et al., 2004; [4]Martins-Bessa et al., 2021. ND: not determined

Table 2. Mean (± SD) of the donkey (*Equus asinus*) breeds seminal parameters.

Parameters	Pêga donkey[1]	Catalonian donkey[2]	Andalusian donkey[3]	Martina Franca donkey[4]
Gel free semen volume (mL)	47.2±28.6	56.61±23.18	80.6±11.1	90±43.4
Gel volume (mL)	71.7±54.8	ND	ND	107.2±41.6
Total motility (%)	84.2±6	68.40±16.59	90.2±2.7	81.9±3.7
Progressive motility (%)	74.4±7	ND	70.1±4.1	76.6±4.8
Sperm concentration (10^6 per mL)	253±91.2	280.88±228.94	259.4±37.6	350.4±139.7
Total abnormalities	7.9±3.0	18.99±8.62	12.2±2.1	ND
pH	ND	7.77±0.35	7.2±00	7.6±0.2
VCL (m/s)	ND	80.20±51.72	106.6±0.2	115.9±14.2
VSL (m/s	ND	49.80±43.18	78.5±0.3	195.9±27.7
VAP (m/s)	ND	59.39±43.04	94.4±0.2	136.8±12.5
LIN (%)	ND	60.50±28.79	73.3±0.2	60.8±5.4
STR (%)	ND	79.21±24.50	82.2±0.2	89±40

[1] Canisso et al., 2010; [2] Miro et al., 2005; [3] Dorado et al., 2013; [4]: Contri et al., 2010. ND: not determined

Table 3. Effect of different extenders in post thawing donkey semen cryopreservation

Author	Extender	Breed	Concentration	Hours post-thaw	Progressive motility (%)	Total motility (%)	VAP (μm/s)
Álvarez et al. (2019)	Lactose–jenny colostrum extender	Zaragoza, Spain	50% of lactose, 20% of jenny colostrum, 25% of Glucose–EDTA and 5% DMF.	0	ND	58.3	50
Oliviera et al. (2014)	CLC	Pega	3mg	0	49.2 ± 2.7	81.1 ± 2.9	89.6 ± 2.7
Rota et al. (2012)	Eg-INRA96	Amiata	2.8 mL	0	33	53	107
				1	31	43	100
				2	21	31	85
Rota et al. (2012)	Gly-INRA96	Amiata	4.4 mL	0	32	49.5	110
				1	28	42	95
				2	21	32	83

CLC: Cholesterol-loaded cyclodextrin; Eg:Ethylene glycol; Gly: Either glycerol, ND: not determined

Sperm quality and cryopreservation

Puberty is the first release of fertile sperm. This is well correlated with the development of gonads, which the donkeys are under breeding conditions and environmental factors. It has been demonstrated that puberty, by the presence of spermatozoa in the epididymis, is observed more early in the low density of donkeys having a high population density than the other having low population density of animals (Choquenot, 1991). The success of the cryopreservation process is conditioned by the survival of the plasma membrane of the sperm, the acrosome, and the mitochondria (Sieme et al., 2015). Indeed, the optimal conditions of cryoconservation are aimed to provide the physicochemical properties during freezing, as well as the optimal cooling rates, maintaining temperatures, and warming rates for the sperm (Parks and Graham, 1992).

Many studies have aimed to describe the reproductive capacities of the different breeds in different localities (Table1). Carluccio et al. (2013) reported that the percentage of viable spermatozoa in the donkey's ejaculates is much higher compared with values reported for the horse, (80% *vs.* 55 to 65%). Other published results suggest that vitamin C and E could increase the quality of frozen sperm acting on sperm viability, acrosome cell membrane integrity, and mitochondrial activity. Also, the fusion of both vitamins provides a better effect than the single addition (Yu et al., 2019). According to Álvarez et al. (2019), the use of an extender containing lactose-jenny colostrum can be successfully used for donkey semen cryopreservation and could effectively improve donkey sperm qualities after freezing-thawing (Table 3).

Rota et al. (2012) reported that the use of either glycerol or ethylene glycol as a cryoprotectant after thawing and dilution with seminal plasma appears to improve fertility and pregnancy rates of inseminated jennies. While Oliviera et al. (2014) demonstrated that the use of cholesterol-loaded methyl-b cyclodextrin on donkey semen before cryopreservation increased the viability of thawed spermatozoa (Table 3).

The vitrification technic by direct exposure of sperm to liquid nitrogen is a method of biotechnology related to reproduction and seems increasing in popularity as an alternative to conventional freezing. Although, Hidalgo et al. (2020) showed that donkey sperm could not be vitrified using only glycerol as permeable cryoprotectant agents. Vitrification using non-permeable cryoprotectant agents (sucrose 0.1 M and BSA 5%) enhanced sperm motility and viability after warming.

In another study, donkey semen diluted in skimmed milk glucose showed superior sperm motility than lactose - egg yolk. The pregnancy rate for mares inseminated with semen diluted in skimmed milk glucose was higher than that obtained using lactose - egg yolk (56.52% *vs*. 4.76%, respectively) (Carvalho, et al., 2017**)**. Moreover, total cholesterol (mg/dl), total protein (g/dl) and triglycerides (mg/dl) were significantly higher in donkey's seminal plasma (74.65±9.70, 3.63±0.50 and 61.72±7.3, respectively) than in horse (64.72±10.23, 1.25±0.19 and 39.57±8.35, respectively) (Pal et al., 2009). These results could be explained by the fact that donkeys tend to be more fertile than horses, presenting a conception rate of 78% compared to a mare's average of 65% (Hagstrom, 2004).

CONCLUSION

Equine professionals recognize that donkey is an abandoned animal source, and their value is often underestimated in the equine world. Although the present mini-review has focused on the reproduction in male donkeys, it would be also needed to mention other important physiologic aspects in order to ensure genetic diversity. Reproductive physiology is a key essential to undertake research in safeguarding the genetic inheritance of endangered animal species. Therefore, it is very important to review some aspects of reproduction to understand asine breeding management. The donkey belongs to the family of Equidae, and exhibits some similar features of reproduction to horse. It appeared that the seasonality is well marked in donkeys, with other factors besides photoperiod and ambient temperature influencing the asine reproduction. Previous studies revealed that there is variability in sperm quality and production in donkeys around the world.

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S.A; writing—original draft preparation: S.A., A.A.; supervision: A.A. All authors read and approved the final manuscript.

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