Case Report

Plugged Ampullae in a Donkey Stallion (*Equus asinus*)

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**A B S T R A C T**

The donkey jack sex glands are larger than stallions, responsible for producing most part of seminal plasma and the second fraction of ejaculate, along with epididymis tail. Plugged ampullae occur by sperm accumulation obstructing the lumen, inducing decrease in sperm quality and may cause azoospermia. In this study, a Pega breed donkey jack, aging 4 years, was evaluated for breeding soundness evaluation due to a sudden decrease in semen parameters and low fertility rates. Palpation, measurements, and ultrasonic examinations of testes were normal; however, rectal palpation revealed increased volume of ampullae and deferent duct, and the transrectal ultrasonography revealed distended ampullae with hyperechogenic material in the ampullae lumen. After ampullae massage, the semen was collected with artificial vagina for evaluation, resulting in high concentrated semen (1.46 × 10⁹ spermatozoa/mL) with low motility (5%), 14% of major defects, and 57% of minor defects. Plugged ampullae were suggested, and the treatment was performed by ampullae massage per rectum and three consecutive semen collections associated with the parenteral use of oxytocin 20 IU iv, aiming to discharge the semen accumulation. Daily regimen of semen collection was recommended during 10 days, and after this time, semen was collected at least three times a week. The semen parameters restored to normal (80% motility) after 30 days. The donkey jack returned to the breeding season with a regimen of 3 days a week of semen collection.

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1. Introduction

Accessory sex glands are formed by a paired ampullae, paired seminal vesicles, a single bilobed prostate, and paired bulbourethral glands [1], responsible for producing the most part of seminal plasma. The bulbourethral glands and prostate were responsible to produce the prespermatic fraction (first part of ejaculate), which has the urethra cleaning function and lubrication. The second fraction (rich in spermatozoa) comes from the epididymis tail, deferent ducts, and ampullae. The seminal vesicles are responsible for the last fraction containing the gel that carries sperm left in the urethra [2–4].

Accessory sex glands in donkey stallions (jack) are similar, but larger in dimensions when compared with equine stallion glands [5]. The stallion’s ampullae measure 1–2 cm of diameter [6,7], whereas the donkey’s average 2–4 cm [8]. The fluid produced by donkey ampullae (19–79 mL) is higher than stallion’s secretion and have more ergothioneine in the composition [9]. In asses, the ampullae is shaped cylindrical, located 20 cm cranial from the anus and dorsolateral to the urinary bladder, and characterized by ultrasonic as lengthened hypoechoic mass and lumen with few anechoic content [8].

During andrological examination in stallions, the sexual glands evaluation can support the diagnosis of several reproductive problems [1], being able to help in the differentiation of azoospermic cases [10]. The ampullae lumen obstruction due to spermatozoa accumulation, also denominated spermiostasis or plugged ampullae, occurs when the blockage is complete and bilateral and thus the affected stallion ejaculate azoospermic semen. This condition also can be present as sporadic form, inducing decrease in sperm motility and impaired morphology. Animals with large...
tests and high spermatozoa production are more susceptible to this disease [7].

In the literature, although this condition is well characterized, few cases were reported in stallions and none in donkeys. Based on the reviewed literature and on the best of our knowledge, the present report is the first description of plugged ampullae in donkeys.

2. Case Detail

2.1. History

A Pèga breed donkey jack, aging 4 years, weighing 350 kg, with adequate body conditions (score 7.0) [11] was attended for andrological examination in a stud farm located in Jundiaí city (Latitude 23° 11' 11” S and Longitude 46° 53' 03” W) São Paulo State, Brazil. The donkey received 4 kg/day of concentrated balanced grain, alfalfa hay, water and mineral salt ad libitum, remained loose in piquet during day and stabled at night.

The donkey was performing his first breeding season (2014/2015) and had normal seminal parameters (motility 80% and 8.2 × 10^9 total sperm) evaluated before start, showing normal condition for the species [12,13]. The reproductive management was a controlled natural mating, and a normal fertility persisted during the first 2 months. However, after 2 months, from the start of breeding season, some negative pregnancy diagnoses were observed, and another semen evaluation was carried out, where a sudden decrease in semen parameters was observed, showing decrease in sperm motility and reduction in sperm concentration (1.4 × 10^9 total sperm).

2.2. Clinical Findings and Diagnosis

After complete physical examination of the animal, no clinical or musculoskeletal abnormalities were found. For andrological examination, the donkey was restrained in stock and sedated with xylazine (Sedomin 10%, König, Brazil) 0.3 mg/kg iv. Palpation and measurement (length × width × height) of testicles (right: 9.5 cm × 6.0 cm × 6.5 cm; left: 9.0 cm × 5.5 cm × 6.5 cm) and epididymis (right: 3 cm × 3 cm; left: 3 cm × 3 cm) were performed, and no abnormal differences were observed [14–16]. The testes were in normal position and had normal consistency, absence of pain sensitivity, and mobility within scrotum. Scrotal and testicular ultrasonography did not reveal any abnormalities [17].

Transrectal palpation was performed for accessory glands examination, and an increase in ampullae volume was observed. The transrectal ultrasonographic examination revealed enlargement in total diameter (32 mm), wall thickness, and lumen with hypoechoic mass collection on both ampullae and deferent duct (Fig. 1). Bulbourethral glands, prostate, and vesicular gland presented no alterations [7,8].

After ultrasonography, the ampullae manipulation per rectum was performed, and then semen was collected by artificial vagina (Botupharma, Botucatu, Brazil). Macroscopic analysis revealed ejaculate with 10 ml volume, suis generis odor, and white color with condensed milky appearance, and the sperm concentration was performed with Neubauer chamber resulting in 1.46 × 10^9 spermatozoa/ml, value three to five times higher than expected for the species [13,16,18].

A semen aliquot was added to a buffered formalin solution 10% for wet mount preparations to analyze the spermatozoa morphology by differential interference contrast microscopy (Leica Microsystems, Wetzlar, Germany), which revealed 14% of major defects and 57% of minor defects, especially tailless heads (35%), bent tails (13%), and distal protoplasmic droplets (14%). Furthermore, the total semen was diluted in skimmed milk diluent (BotuSemen Special, Botupharma Botucatu, Brazil) at a 5:1 ratio (diluent:semen) for sperm kinetic evaluation, which showed 5% of total motility (0–100%) [19]. A thin smear of semen was stained with Dip Quick (Instant Prov, New Prov, Brazil) and analyzed by bright-field microscopy (Primo Star, Zeiss, Germany) using a × 1000 magnification to look for cellularity, but no polymorphonuclear cells was observed. An ampullary spermiostasis was suspected based on these initial clinical observations.

2.3. Treatment

Ampullae massage was conducted per rectum one more time according to indications for stallions [7], and then three consecutive semen collections were accomplished, using oxytocin 20 IU iv (Ocitocina HC, Hertape Calier, Brazil) before collection. However, improvements in semen quality were not observed in this time, and sperm concentration decreased in each collection (Table 1).

Donkey was returned to stud with a recommendation to collect semen at least three times a week and in a month; semen parameters were normal again (Table 2).

Donkey was returned to stud with a recommendation to collect semen at least three times weekly, even in nonbreeding season, to avoid the return of ampullae disorder. The animal was accompanied during the following 2 months, and no recurrence was noted. The seminal parameters remained adequate (80% motility and 4.2–7.4 × 10^9 total sperm/collection), as well as conception rates per cycle (72%) during the rest of breeding season.

Table 1: Semen parameters in the three consecutive collections after ampullae massage and oxytocin injection before each collection in a donkey jack with spermiostasis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Collection 1</th>
<th>Collection 2</th>
<th>Collection 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Sperm concentration/mL</td>
<td>2.5 × 10^9</td>
<td>720 × 10^6</td>
<td>340 × 10^6</td>
</tr>
<tr>
<td>Total sperm concentration</td>
<td>22.5 × 10^9</td>
<td>7.9 × 10^9</td>
<td>4.0 × 10^9</td>
</tr>
</tbody>
</table>

Fig. 1. Ultrasonographic image of plugged ampullae. (A) Overall enlargement and hyperechoic mass within the lumen; (B) enlargement of wall thickness.
3. Discussion

Based on the literature and on the best of our knowledge, the present report is the first description of plugged ampullae in donkey jack, and the clinical signs are very similar with stallions’ condition. In both species, the ampullary glands are tubulo-alveolar opening direct in lumen, composed by tunica mucosa, tunica submucosa, tunica muscular, and tunica serosa [20]. During ultrasonographic examination, the glandular size increase when the jack is sexually stimulated and return to normal after ejaculation, likewise stallions [5].

In stallions with plugged ampullae disorder, the ultrasonographic examination showed distention of glands lumen filled with hypoechoic or mixed echogenicity fluid [7,21], and even with hyperechoic material and parenchyma with heterogeneous aspect [22]. It is not known if the donkey presents variation in the aspect of material that obstructs the ampoule, and in the present case, it was observed hyperechoic content.

On account of the ampullae obstruction in stallions, the sperm concentration in the ejaculate can vary broadly, and as the condition progresses, the semen quality decrease due to the extended period storage in extragonadal ducts exposure to body temperature, with higher proportion of tailless spermatozoa head, bent tails, and distal prostatic droplets [7,22], similar to initial description of donkey jack case. However, some plugged ampullae cases can cause azoospermia by total ampullae obstruction [7,21]. To distinguish if the azoospermia derive from ampullae lumen obstruction or testicular disorder, alkaline phosphate (SPAP) concentration can be measured in semen because the enzyme is only produced in testicles and epididymis. In the present report, SPAP dosages could not be performed, because azoospermia was not observed in this jack donkey, probably because it did not have a total ampullae obstruction; however, it did not impair the final diagnosis and treatment. According to McCue [23], after collection of a stallion diagnosed with ampullae obstruction and semen evaluation, less than 30% of the spermatozoa were morphologically normal, which corroborate with our finding, as the total defects add up 71%. Klewitz et al. [21] also reported an 80% proportion of abnormal spermatozoa, mainly midpiece fracture, with 34.8% of membrane-intact spermatozoa and 3.5% of acrosome reacted, measured by cytometry. In another study, Strauch et al. [24] reported that the first five ejaculates from a stallion with ejaculatory failure, diagnosed with ampullae blockage, revealed necroospermia and teratoospermia, which decrease after the sixth semen collection.

The donkey semen analysis demonstrated high concentration of spermatozoa and total number of spermatozoa, as well as low motility before treatment with succeeding collections, which resulted in seminal parameters improvement, similar observed in stallions by McCue [25]. A resembling condition was observed in bulls, which accumulated senescent semen in ampullae and last portion of epididymis, considered a permanent disorder as a consequence of recurrent semen accumulation after a month of sexual rest. The treatment consists in regular semen collection, and it requires a week for the semen parameters return to normal.

Furthermore, affected bulls present high percentage of detached spermatozoa heads similar to donkey jack and stallions [26].

The prescribed treatment for the donkey with ampullae massage, oxytocin application prior collections, and successive semen collections was established according to the stallions treatment in the literature, because of the similar physiology and anatomy [7,21—24]. The positive results, improving semen parameters after treatment, support this conception.

In conclusion, this first report demonstrated that the donkey is susceptible to ampullae obstruction as stallions due to the physiological and anatomical similarities. Also, the same treatment can be prescribed successfully and continuously to prevent recurrences.

References