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Case Report

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

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ABSTRACT

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 Pêga 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 ultrasound examinations of testicles 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 (1.46×10^9 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.

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 ultrasound 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

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testes 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 Pêga 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 restraint 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 hyperechoic 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

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

(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 spermastasis 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).

Daily regimen of semen collections was established during 10 days, using oxytocin before each collection. The sperm concentration did not differ within that period of time. On the fourth day, an increase in seminal quality was noticed, and after 10 days the values almost restored to normal. Subsequently, semen was collected 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 $4.2–7.4 \times 10^9$ 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.

Parameter	Collection 1	Collection 2	Collection 3
Motility	10%	10%	10%
Sperm concentration/mL	2.5×10^9	720×10^6	340×10^6
Total sperm concentration	22.5×10^9	7.9×10^9	4.0×10^9

Table 2
Semen parameters of donkey jack with spermiostasis during the first month after treatment.

Parameter	Day 2–4	Day 4–9	Day 10	Day 30
Motility	10%	40%	70%	80%
Sperm concentration/mL	$290–370 \times 10^6$	$290–370 \times 10^6$	310×10^6	330×10^6
Total sperm concentration	$4.2–7.4 \times 10^9$	$4.2–7.4 \times 10^9$	5.6×10^9	5.2×10^9

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 tubule-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 protoplasmic 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 necrospermia and teratozoospermia, 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

[1] Weber JA, Woods GL. Transrectal ultrasonography for the evaluation of stallion accessory sex glands. Vet Clin North Am Equine Pract 1992;8:183–90.
[2] Amann RP, Graham JK. Spermatozoa functional. In: McKinnon AO, Voss JL, editors. Equine reprod. 1st edn. Philadelphia: Lea and Febiger; 1993. p. 715–45.
[3] Magistrini M, Lindeberg H, Koskinen E, Beau P, Seguin F. Biophysical and 1H magnetic resonance spectroscopy characteristics of fractionated stallion ejaculates. J Reprod Fertil Suppl 2000;56:101–10.
[4] Kareskoski M, Katila T. Components of stallion seminal plasma and the effects of seminal plasma on sperm longevity. Anim Reprod Sci 2008;107:249–56.
[5] Contri A, Tosi U, De Amicis I, Veronesi MC, Panzani S, Carluccio A. Ultrasonographic evaluation of sexual glands before and after ejaculation in the jackass. Vet Res Commun 2008;32:135–7.
[6] Pozor MA, McDonnell SM. Ultrasonographic measurements of accessory sex glands, ampullae, and urethra of normal stallions of various size types. Theriogenology 2002;58:1425–33.
[7] Varner DD, Schumacher J. Abnormalities of the accessory sex glands. In: McKinnon AO, Squires EL, Vaala WE, Varner DD, editors. Equine reprod. Second 2011. p. 1113–8.
[8] Kandiel MMM, El Shafey AA. Ultrasonographic appearance and echo-pattern characterization of donkeys' internal reproductive organs received: accepted. J Adv Vet Res 2017;7:39–46.
[9] Mann T, Leone E, Polge C. The composition of the stallion's semen. J Endocrinol 1956;13:279–90.
[10] Blanchard TL, Brinsko SP, Varner DD, Love CC. How to investigate azoospermia in stallions. AAEP Proc 2009;55:331–5.
[11] Henneke DR, Polter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983;15:371–2.
[12] Gastal MO, Henry M, Beker AR, Gastal EL. Effect of ejaculation frequency and season on donkey jack semen. Theriogenology 1997;47:627–38.
[13] Canisso IF, Carvalho GR, Morel MCGD, Guimarães JD, McDonnell SM. Sexual behavior and ejaculate characteristics in Pêga donkeys (Equus asinus) mounting estrous horse mares (Equus caballus). Theriogenology 2010;73:56–63.
[14] de Morais RN, Mucciolo RC, Viana WG. Biologia reprodutiva de jumentos. I. Biometria testicular E comportamento sexual durante a colheita de sêmen. Braz J Vet Res Anim Sci 1993;30:47–50.
[15] Canisso IF, Souza FA, Carvalho GR, Guimarães JD, Silva EC, Lima AL. Alguns aspectos fundamentais do exame clínico andrológico de jumentos (Equus asinus). Rev Bras Reprodução Anim 2008;32:233–9.
[16] Canisso IF, de Carvalho GR, da Silva EC, Rodrigues AL, Ker PG, Guimarães JD. Alguns aspectos biométricos do aparelho genital externo de jumentos doadores de sêmen da raça Pêga. Cienc Rural 2009;39:2556–62.
[17] Love CC. Ultrasonographic evaluation of the testis, epididymis, and spermatic Cord of the stallion. Vet Clin North Am Equine Pract 1992;8:167–82.
[18] de Morais RN, Mucciolo RG, Viana WG. Biologia reprodutiva de jumentos. II. Características físicas e morfológicas do sêmen. Braz J Vet Res Anim Sci 1994;31:49–57.
[19] Kenney RM, Bielanski W. Society for theriogenology manual for clinical fertility evaluation of the stallion. J Soc Theriogenology 1983;9:100.
[20] Abou-Elhamd AS, Salem AO, Selim AA. Histological and histochemical studies on the ampulla of the deferent duct of donkey (Equus asinus). J Adv Vet Res 2012;2:261–70.
[21] Klewitz J, Probst J, Baackmann C, Keppler S, Ortgies F, Heberling A, et al. Obstruktion der Samen leiter ampullen (plugged ampullae) als Ursache einer Azoospermie bei einem Hengst. Pferdeheilkunde 2012;28:14–7.
[22] Turner RM. Problems of the accessory sex glands. Proc Soc Theriogenology Annu Conf 2011:7–8.
[23] Mccue PM. "Sperm accumulation" in a stallion. Equine Reprod Case Files 2013:1–4.
[24] Strauch AM, Garret EF, Hayna J, McCoy AM, Lascola KM, Ferrer MS, Canisso IF. A challenging case of ampullary blockage in a geriatric American Saddlebred stallion. Clin Theriogenology 2017;9:500.
[25] McCue PM, Scoggin CF, Moffett PD, Burden CA, Ferris RA. Spermiostasis in stallions: a retrospective study of clinical cases. J Equine Vet Sci 2014;34:47.
[26] Barth AD. Sperm accumulation in the ampullae and cauda epididymides of bulls. Anim Reprod Sci 2007;102:238–46.