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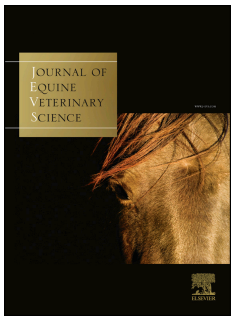

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Effect of pergolide therapy on semen parameters in a stallion with pituitary pars intermedia dysfunction

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Abstract

A 23 year-old Warmblood stallion with pituitary pars intermedia dysfunction (PPID) was treated with 0.002 mg/kg body weight pergolide orally once a day, starting in July. Semen collections during five consecutive days each were performed before starting and at 90 days after initiation of the therapy in order to assess the effect of the therapy on semen volume, concentration, total sperm count, motility and viability. The only changes observed were lowered semen volume compensated by increased semen density resulting in an equal total sperm count. Possible causes for these observations include altered prolactin levels, the influence of season, or pergolide acting directly on the ejaculation process. This case report provides first evidence, that pergolide therapy in PPID may alter semen parameters, but these changes are unlikely to affect fertility. However this observation in a single individual must be confirmed with experiments on multiple stallions.

Keywords: horse; cushing; treatment; male; semen quality

Introduction

Pituitary pars intermedia dysfunction (PPID) or equine Cushing syndrome is an endocrine disorder which mostly occurs in aged horses without a breed predisposition [1-2]. Hypertrichosis is the pathognomonic sign of PPID (55% - 80% of cases) [1], but other clinical signs associated with PPID include polyuria and polydypsia, chronic infections, laminitis, catabolism of muscles i.e. in the back and abdominal region [2], as well as disturbances of the reproductive functions with impaired fertility [1, 3]. These reproductive problems have been observed in mares, namely inhibition of normal ovarian activity, small sized follicles, absence of a corpus luteum, flaccid uterus as well as problems to maintain a normal pregnancy [4]. Case reports suggest that pergolide treatment of PPID can restore reproductive function in those animals [4-5]. In contrast to these observations in mares, the effects of pergolide treatment on the male reproductive system have so far not been documented. In a recently published case report no abnormalities were found in routine semen examinations of a 21-year-old PPID-affected stallion and no histopathological alterations were seen in the testicles. However treatment effects of pergolide could not be investigated due to anticipated death of the stallion [6].

The pathogenesis of PPID is not fully understood. It is hypothesized that PPID mainly results from oxidative stress and neurodegeneration in the dopaminergic neurons of the hypothalamus causing a decrease in dopamine production. The loss of the negative control by dopamine in the pituitary pars intermedia leads to an excessive production of propiomelanocortin-derived peptides [5, 7]. It is still unknown which specific endocrine changes lead to which clinical signs observed in horses affected with PPID, and more specifically, to which potential disturbances of the reproductive system. Prolactin seems to play a major role in humans suffering from diseases associated with the pituitary gland, but whether similar effects are also present in horses with PPID is unknown [8-9].

In PPID, pergolide, a long-acting dopamine agonist, is the medical therapy of choice [3]. Positive response to medication is evidenced by improvement of clinical signs and a decrease of plasma adrenocorticotropic hormone (ACTH) concentration [3]. There is, to the authors' knowledge, no information regarding the effect of pergolide on the equine male reproductive system. This report describes the case of a stallion with PPID and compares the sperm quality and quantity before and after initiation of a therapy with pergolide.

Material and methods

Animal, anamnesis, clinical/ endocrinological findings and treatment

A 23 year old Holstein stallion weighing 571 kg was presented with signs of hypertrichosis and a history of delayed shedding of the winter coat. Furthermore, he showed slight axial musculature atrophy and a cresty neck. Plasma ACTH measurement was performed in February because of suspected PPID. A venous EDTA blood sample was collected and immediately centrifuged (3000g; 10 min) in a special EDTA-Plasma stabilisation tube (ACTHstab-Mikrogefäss, ALOMED, Radolfzell-Böhringen, Germany). The plasma was then collected and sent to the laboratory (ALOMED, Radolfzell-Böhringen, Germany), where plasma ACTH concentration was measured using chemiluminescence immunoassay.

The ACTH plasma concentration was 87 pg/ml, suggesting a high probability for PPID in this horse [7, 10]. The stallion was treated with 0.002 mg/kg body weight pergolide (Prascend®, Boehringer Ingelheim, Basel, Switzerland) orally once a day starting the 18th July until his death 3 years later. A second blood analysis six months after initiation of pergolide therapy using the same approach revealed a plasma ACTH concentration (37 pg/ml) within the normal range base on the laboratory references [10], but still slightly above the upper threshold described in the study of McGowan et al. in 2013 (29.7 pg/ml) [7].

Semen evaluation

Two semen evaluation series took place from 9th to 13th July before and from 15th to 19th October 90 days after initiation of the pergolide treatment in order to compare the effects of the medication on semen parameters. Before each semen evaluation series, semen collections were performed one time per day for five consecutive days in order to deplete and stabilize the extragonadal sperm reserves as described by Thompson et al. [11]. After a break of one day, semen was again collected daily once during five consecutive days, followed by a comprehensive semen evaluation of each ejaculate.

Ejaculates were collected using a dummy and an artificial vagina (type Avenches, Swiss National Stud, Avenches, Switzerland). Immediately after collection and removal of the gel fraction, the volume of the ejaculate was determined using a graduated container, the sperm concentration and viability (integrity of the sperm plasma membrane) were evaluated using a nucleocounter (NucleoCounter® SP-100™ Sperm Cell Counter, ChemoMetec A/S, Allerød, Denmark [12]) and total sperm number was calculated. Total and progressive motility were assessed in 10 µl raw semen diluted with 20 µl INRA 96™ (IMV Technologies, L'Aigle, France) with a computer assisted sperm analyzer (HTM-IVOS, Version 12, Hamilton Thorn, Beverly, Massachusetts, USA) using a 20 µm standard count analysis chamber (Art. Nr. SC 20-01-C, Leja, Nieuw-Vennep, Netherlands) and standardized threshold values for stallion semen.

Statistics

Statistical analysis was carried out using NCSS software (NCSS 2007, Statistical Solutions, Saugus, USA). Semen volume, concentration, total sperm count, motility and viability were normally distributed and parametric paired t-tests were performed in order to assess the effects of treatment. Results were expressed as means ± standard deviation (SD) of the

116 average recordings before and after treatment. Values were considered to be statistically
117 significant at a probability level of $P < 0.05$.
118
119 **Results**
120 After 90 days of therapy, improved hair coat quality and body condition were observed. No
121 adverse side effects were witnessed during the course of the treatment. The stallion did not
122 exhibit any behavioral changes during semen collection. Results of the spermatological
123 analysis are illustrated in Table 1. The volume of the ejaculates was significantly higher in
124 the samples collected before pergolide treatment compared to the samples collected after
125 initiation of the therapy ($P = 0.003$). Semen concentration showed a tendency to be increased
126 during the therapy ($P = 0.06$), while the other parameters showed no changes.
127
128 **Discussion**
129 Adequate management of aged stallions is crucial for maintaining their reproductive capacity
130 as long as possible. The present report describes standard variables of semen assessment in a
131 single PPID-affected breeding stallion treated daily with pergolide for 90 days. Our
132 observations indicate that the changes in semen parameters that occurred during the thirteen
133 week treatment period are unlikely to affect reproductive capacity. The only alteration
134 observed was a lowered semen volume compensated by an increased semen density leading
135 to an equal total sperm count.
136 Impaired female reproductive capacity associated with PPID has been reported [3-5], but the
137 specific endocrine cause of this observation is unknown. Similarly, the effect of the treatment
138 of choice, pergolide, on fertility, pregnancy and lactation has only been described in two
139 reports of single cases [4, 13]. Fertility problems associated with PPID in aged stallions are
140 much less recognized, likely since the number of stallions used in reproduction is much

smaller than that of mares. Nevertheless, there is strong interest in evaluating effects of the disease itself and the associated therapy on reproductive capacity of affected males [6].

PPID is a disease affecting the hypothalamic-pituitary-adrenal axis leading to alterations in the release of several hormones including dopamine, ACTH, melanocyte-stimulating hormone or corticotropin-like intermediate peptide [5]. Reproductive hormones like prolactin, gonadotropin-releasing-hormone (GnRH), luteinizing hormone (LH) or follicle stimulating hormone (FSH) produced in the pituitary gland or testosterone produced in the adrenal gland are all potentially altered in horses with PPID and these changes might result in a decreased reproductive capacity in aged mares and stallions. Furthermore, pergolide, a dopamine agonist used in the treatment of PPID, might also influence levels of those hormones, again potentially altering the reproductive capacity in stallions.

In human medicine, signs of reduced reproductive capacity have been described in males with a similar disease called Cushing's syndrome (loss of libido, loss of erectility, decreased testosterone level) [14]. In one case, high cortisol levels impaired testicular function and influenced testosterone concentrations [14]. In humans, pituitary adenomas often represent prolactinomas inducing hyperprolactinaemia [8]. The important role of prolactin in fertility is well documented: Prolactin acts as a modulator on the hypothalamus-pituitary-gonadal axis [15], and its secretion is regulated by the pituitary portal circulation through dopamine [8]. It has been reported in humans that high levels of prolactin have negative effects on testosterone levels through inhibition of GnRH leading to decreased LH and FSH secretions [15], and can impair spermatogenesis, but also decrease libido and provoke erectile dysfunction and prostate hyperplasia [8-9, 15]. Treatment with the dopamine type 2 (D2) selective-agonist cabergoline has been used to re-establish normal levels of prolactin [9, 15] and has been shown to provide a beneficial effect on the sperm quality, specifically regarding the number of spermatozoids and their motility [15].

Based on a study by Thomson et al. [11], prolactin also plays a role in stallion fertility, in particular regarding accessory gland functions. It seems to have an effect on the seminal ejaculate gel-free volume. The lower volume values observed in our case could therefore be explained by a decrease of prolactin blood levels. However, contrary to findings in humans, a previous study found no significant effects of the D2-agonist bromocriptine on spermatozoid numbers in horses [11]. Based on this observation, pergolide therapy would not be expected to influence spermatozoid numbers, at least not by way of an effect on prolactin. In men, results were evaluated after prolonged treatment, e.g. 12 – 24 months [15], and showed an earlier improvement of ejaculate volume and sperm count than of all other parameters [3]. In our case, semen quantity and quality was assessed after a comparatively shorter pergolide treatment interval. The observation period of 90 days was chosen because it largely encompassed the duration of spermatogenesis in stallions and the time of 6 to 8 weeks needed to observe clinical improvement [16-17].

A further explanation for the observed decrease of ejaculate volumes could be a direct influence of pergolide on the D2-receptor of the central or peripheral nervous system influencing the ejaculatory process [11]. Many mammalian (including equine) spermatozoids have also been shown to exhibit D2 receptors which are sensitive to dopamine and dopamine agonists [18-19]. According to different studies in human and equine semen, dopamine agonists have a negative influence on semen motility [18-19]. We cannot confirm such an effect in the single case we have documented.

Finally, the changes that we have observed were associated with the pergolide treatment period, but pergolide may not be the cause of these alterations. The endocrine system is affected by many factors like environment, season, nutrition and age [20-22]. These variables could all influence the paracrine and autocrine modulation [21]. It has been demonstrated that during the breeding season stallions have increased testicular function [22-23]. In our case,

first sperm collection series was performed in July (summer and end of breeding season) while the second collection series took place in October (autumn and no breeding season). Observed decrease of semen volume and by trend higher concentration go in line with our reference values of 10 Warmblood stallions evaluated in a former study in the same stud farm (41.2 ml and 270.0 x 10^6 sp/ml in summer *vs.* 36.3 ml and 323.1 x 10^6 sp/ml in autumn [22]). This case report provides first evidence, that pergolide therapy in PPID may alter semen parameters, but these changes are unlikely to affect fertility. However this observation in a single individual must be confirmed with experiments on multiple stallions during different seasons of the year and variable treatment intervals In addition, it would be interesting to complement the variables reported here by also variations of prolactin, LH, FSH and testosterone.

Authors' declaration of interests

None of the authors have any financial and personal relationships with other people or organizations that could inappropriately influence the study.

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Table 1: Summary of the means ± standard deviations (SD) of various semen parameters collected over a period of 5 days each prior and after 90 days of oral pergolide treatment in a stallion.

Parameter	Before pergolide treatment		During pergolide treatment	
	mean	± SD	mean	± SD
Volume (ml)	62.2	9.4[a]	33.4	12.1[b]
Concentration (x 10^6 sp/ml)	139.6	36.9	236.0	90.20
Total sperm count (x 10^6 sp)	8'766	2'895	7'484	2'336
Total motility (%)	83.8	6.4	87.6	2.6
Progressive motility (%)	58.2	5.8	61.4	4.4
Viability (%)	88.0	2.0	87.8	0.8

Means with different superscripts (a, b) are significantly different ($P < 0.05$).