USE OF A DESLORELIN IMPLANT FOR INFLUENCING SEX HORMONES AND MALE BEHAVIOUR IN A STALLION – CASE REPORT

Susanne SCHÖNERT1*, Martina REHER2, Achim D. GRUBER3 and Bianca CARSTANJEN1,4

1Equine Clinic: Surgery and Radiology, Department of Veterinary Medicine, Freie Universität Berlin, Oertzenweg 19b, 14163 Berlin, Germany; 2Virbac Animal Health, Bad Oldesloe, Germany; 3Institute of Veterinary Pathology and Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany; 4Department for Large Animal Diseases, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

(Received 29 May 2011; accepted 11 October 2011)

This case report describes the use of a subcutaneously applied 4.7-mg deslorelin acetate implant in a three-year-old Arabian crossbred stallion showing unwanted strong male behaviour. Following deslorelin acetate implantation the stallion showed a short transitional increase in male behaviour. A ‘gelding-like’ behaviour was noted 15 days (D15) after treatment. The horse was surgically castrated at the owners request at D52 after treatment. Serum testosterone, oestradiol-17β and oestrone sulphate values decreased after deslorelin acetate implantation, but serum LH and FSH levels remained unchanged. Histopathological analysis of both testes and sperm analysis revealed a reduced spermatogenesis at D52. The testicular volume decreased after treatment. The use of a subcutaneously applied deslorelin acetate implant might be a promising tool to change the behaviour of aggressive stallions.

Key words: Horse, stallion, male aggressive behaviour, castration

The intention to castrate a stallion can arise for various reasons (Stout, 2005), such as difficulties in handling as sport or pleasure horse or medical causes. Surgical castration is a procedure commonly performed in stallions but is associated with a high complication rate (Stout, 2005). Various minimally invasive methods, such as the administration of gonadotropin-releasing hormone (GnRH) agonists, GnRH antagonists, immunisation against GnRH as well as the use of GnRH toxin conjugates, have been described as methods to temporarily or permanently castrate males of various species (Hinojosa et al., 2001; Malmgren et al., 2001; Kutzler and Wood, 2005; Padula, 2005; Turkstra, 2005; Trigg et al., 2006; Junaidi et al., 2007). Indications for a temporary, non-surgical reduction of
sexual hormones combined with a decrease of typical stallion behaviour are: (i) possible future use of the animal as a breeding horse; (ii) medical reasons not allowing a standard surgical castration, or (iii) the owner’s request for a non-surgical procedure.

Case report

A three-year-old white Arabian crossbred stallion was referred with a history of highly distinctive male behaviour with aggression towards people and other horses. The stallion was kept on pasture and had no direct but sight contact with mares and geldings. He was intended to be used as a pleasure horse. Because it was difficult to train the horse with his intense male behaviour, the owner requested for a possibility to solve the testosterone-related behavioural problems without surgical castration.

On admission at the clinic in May 2008 the stallion was in good general health (whole body weight: 320 kg; height: 151 cm). The results of general clinical examination, blood analysis and biochemical blood values were within the physiological ranges. Both testes were located in the scrotum. Testicles, penis, prepuce and accessory glands were normal on inspection and palpation. On the owner’s request and with the aim of a temporary castration, a 4.7-mg deslorelin acetate implant (Suprelorin®, Virbac Tierarzneimittel GmbH, Germany) was administered subcutaneously into the left side of the horse’s neck. The stallion’s behaviour towards horses and humans as well as his general health status and the site of implant application were monitored closely for a period of eight weeks following the deslorelin acetate implantation (Fig. 1). The day of implantation was defined as day 0 (D0). Venous blood samples were taken at D0, D8, D15, D22, D30, D37, D52, D59 and D66 (Fig. 1) by jugular venipuncture into 10-ml serum tubes (Sarstedt AG & Co., Germany). The blood samples were centrifuged at 3600 × g for 10 min. Until analysis, serum samples were stored in aliquots of 1.8 ml (Cryovial, Simport, Canada) at –80 °C. The samples were analysed using commercially available test kits for quantification of serum testosterone (Testosterone-ELISA, Neogen Corporation, USA), serum oestradiol-17β (Oestradiol-6, Centaur Bayer Corporation, Germany) and serum oestrone sulphate (Oestrone-sulphat-ELISA, Laboklin GmbH & Co. KG, Germany). Serum luteinising hormone (LH) and follicle-stimulating hormone (FSH) concentrations were quantified with an LH and FSH radioimmunoassay, respectively (Harbor-UCLA Medical Center, CA, USA). Additional EDTA-blood samples (5 ml, Sarstedt AG & Co., Germany) were taken at D0 and D52. The samples were analysed for differential count of white blood cells (Coulter Counter Abacus junior vet 5, Fa. Thermo Fisher Scientific, Germany). On D52 the horse was castrated at the owner’s request because of the reappearance of unwanted intense male behaviour on D45. During surgery both ductus deferentes were clamped with a forceps before liga-
tion and transection of the spermatic cord. The *ductus deferentes* were then punctured using an 18G needle (Braun Melsungen AG, Germany) and lavaged with 5 ml Equi Pro (Minitüb Abfüll- und Labortechnik GmbH & Co. KG, Germany). The semen was collected in sterile glass tubes and stored in warm water of 37 °C temperature until analysis for 3 min. The concentration of spermatozoa was evaluated by photometer (Spermacue, Minitüb Abfüll- und Labortechnik GmbH & Co. KG, Germany) and the percentage of motile spermatozoa was assessed by phase-contrast microscopy (Olympus BX41, Olympus Deutschland GmbH, Germany). Twices one hundred spermatozoa were analysed and the previously described supravital staining according to Dott and Foster (1972) was performed. Both testicles were stored in 5% neutral buffered formalin and four samples of each testicle were selected for histological analysis. In addition, three samples of each epididymis were analysed. The samples were embedded in paraffin and 4-μm thick sections were cut and stained with eosin and haematoxylin.

The stallion showed increased male sexual and aggressive behaviour during the first two weeks following deslorelin acetate implantation, but from D15 onward a ‘gelding-like’ behaviour was noticed, e.g. the stallion showed no interest in mares and geldings. However, at D45 the stallion-like behaviour reap-

---

**Fig. 1.** Comparison of serum concentrations of testosterone (nmol/l), oestradiol-17β (pmol/l), oestrone sulphate (nmol/l), FSH (ng/ml) and LH (ng/ml) in the peripheral venous blood of a stallion before (D0) and after subcutaneous application of an implant containing 4.7 mg deslorelin acetate [I: day of deslorelin acetate implantation (D0); II: the horse showed ‘gelding-like behaviour’ (D15); III: the horse showed reappearance of aggressive behaviour (D45); IV: surgical castration (D52)]
peared. During the whole sampling period, the stallion was in good health and the site of implantation appeared physiological. Initially, the implant could be palpated, but was no longer detectable three months after application. Measurement of the testicles was performed at D0 and D52 using a calliper. The size of the left testicle (length × width × height) decreased from 13 × 8 × 6 cm (D0) to 10 × 6.5 × 4.5 cm (D52), and that of the right testicle from 10 × 8 × 5 cm (D0) to 9 × 6.5 × 5 cm (D52). The testicular volume decreased from 217.82 cm³ (D0) to 153.15 cm³ (D52) for the left and from 209.44 cm³ (D0) to 102.10 cm³ (D52) for the right testicle. Both differential blood counts were in the physiological range when compared to healthy untreated horses. The LH and FSH serum concentrations are shown in Fig. 1. After implantation, the serum hormone concentrations decreased until D30 and a slight increase in serum hormone concentration was detected from D37 to D52 (Fig. 1). Compared to the physiological values for age-matched three-year-old stallions a reduced quantity and motility of spermatozoa were measured in the ductus deferens of both testes (Table 1; Johnson et al., 1991). The supravital staining revealed up to 80% of dead spermatozoa (Table 1). The germinal epithelium was markedly atrophic in both testes in all locations examined (Fig. 2A–C). Few maturing spermatocytes were present in the lumen of the testicular canals. When compared to several age-matched stallions (Johnson et al., 1991), the overall spermatogenetic activity was estimated to be 20 to 30%. Both epididymides contained well-structured and matured spermatids in low numbers (Fig. 2A–C). A second finding was a prominent diffuse hyperplasia of Leydig cells in both testes (Fig. 2A–C). All vascular structures of the pampiniform plexus as well as the scrotal dermal and connective tissue structures were normal.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Left testicle</th>
<th>Right testicle</th>
<th>Merkt and Klug, 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive motility</td>
<td>1%</td>
<td>5%</td>
<td>&gt; 50%</td>
</tr>
<tr>
<td>Supravital staining</td>
<td>20 : 80%</td>
<td>50 : 50%</td>
<td>80 : 20%</td>
</tr>
<tr>
<td>Density (million spermatozoa/mL)</td>
<td>66</td>
<td>265</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leydig cells: hyperplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductus epididymidis:</td>
<td>30% live spermatozoa</td>
<td>80% live spermatozoa</td>
<td></td>
</tr>
<tr>
<td>Plexus pampiniformis:</td>
<td>normal</td>
<td>normal</td>
<td></td>
</tr>
</tbody>
</table>

*Acta Veterinaria Hungarica 60, 2012*
Histological examination revealed marked atrophy of the germinal epithelium with reduced maturation of spermiocytes (arrows) (A). The Leydig cells were markedly hyperplastic throughout both testes (B). The epididymides contained regularly structured spermatids without unequivocal reduction in number, compared with control stallions which had wide per se variations in the number and densities of these cells (C). Haematoxylin and eosin (HE) stain, magnification ×200 (A) or ×400 (B, C)
Discussion

GnRH is synthesised in the hypothalamus and is a key regulator of mammalian reproduction. Following a pulsatile release, GnRH stimulates the secretion of the gonadotropins FSH and LH from the anterior pituitary gland (Brinsko, 1996; Roser, 2001; Padula, 2005). FSH stimulates Sertoli cells, which are responsible for spermatogenesis (Roser, 2008). LH induces Leydig cells to produce testosterone, oestrogens and other factors (Roser, 2001). The Leydig cells are also the main location of the P450 aromatase, which is responsible for the transformation of testosterone into oestrogens. Among other functions, testosterone is needed for spermatogenesis (Roser, 2001), 5α-dihydrotestosterone is responsible for the maturation of spermatozoa (Roser, 2008). Testosterone is the precursor of oestrogens (Hejmej et al., 2005), which can be divided into bound oestrogens (oestrone sulphate) and unbound oestrogens (oestradiol-17β). Unbound oestrogens influence all organ systems and are also held responsible for the stallion-like behaviour.

It is well known that in many species GnRH agonists cause a desensitisation of the pituitary towards stimulatory effects of GnRH and a down-regulation of the GnRH receptors (Ludwig, 2008). Deslorelin acetate binds to GnRH receptors and initially causes a stimulation of the pituitary to increase the LH and FSH secretion due to a permanent stimulation. This stimulatory effect initially causes an increase of serum testosterone and oestrogen concentrations. An interruption of the hypothalamus–pituitary pathway due to a delayed degradation of the GnRH agonist at the corresponding receptors follows (Padula, 2005). This leads to a reduced release of LH and FSH (Padula, 2005; Junaidi et al., 2007). The lowered levels of circulating LH cause a reduced stimulation of the Leydig cells with associated reduction of testosterone production (Padula, 2005; Junaidi et al., 2007).

The effects of prolonged administration of GnRH are discussed controversially in stallions. It has been described that the subcutaneous administration of a deslorelin acetate implant in normal stallions and steroid-treated geldings causes an initial short-term stimulation of LH and FSH secretion and a long-term suppression of both gonadotropins (Johnson et al., 2003). Boyle et al. (1991) reported a decrease in the serum concentrations of LH, FSH, testosterone and oestrone sulphate as well as a decrease in daily sperm production without influencing libido following the use of GnRH agonists. Also, in mares a down-regulation following the application of GnRH agonist was observed (Johnson et al., 2002). Other authors believe that GnRH agonists are not suitable for reducing the serum concentrations of gonadotropins, testosterone and oestrogens (Montovan et al., 1990; Stout and Colenbrander, 2004).

Due to the owner’s request and different opinions about the use of GnRH agonists in stallions, a 4.7-mg deslorelin acetate implant was administered to the stallion. The serum concentrations of gonadotropins, testosterone and oestrogens

Acta Veterinaria Hungarica 60, 2012
after application of the implant are presented in Fig. 1. In this case, deslorelin acetate seems to induce a temporary increase of the previously displayed male behaviour, followed by a temporary ‘gelding-like’ behaviour from D15 onward. The observed changes in the serum concentrations of testosterone and oestrogens correspond with the behavioural changes. From D37 onward an increase of serum testosterone and oestrone sulphate concentrations is observed. They are almost certainly related to the rapid degradation of the GnRH agonist at the receptor site. However, a corresponding increase of FSH and particularly LH serum concentrations is not detectable. A rise of LH should have been presentable as it stimulates testosterone synthesis and precedes testosterone release. FSH serum concentrations are relatively maintained due to a long half-life (Roser, 2008). The reason for the absence of the LH peak remains unclear.

Some authors could not obtain a castration-like effect in stallions (Montovan et al., 1990; Stout and Colenbrander, 2004). The increase of stallion-like behaviour in the animal described in this report became apparent from D45 onward, and it correlates, in a delayed manner, with the increase of serum testosterone concentration. This was the reason for surgical castration on D52.

It is debatable whether the decrease of gonadotropins, sexual hormones and male behaviour is incidental or whether the stallions’ sensitivity toward GnRH implants is highly individual.

Interestingly, the Leydig cell number was markedly increased compared to physiological values for the Leydig cell volume per testis. This suggests a compensatory attempt as a response to the anti-androgen effect of the deslorelin acetate. The reduced oestrogen levels in the case described may be the result of a decreased synthesis and release of testosterone (Fig. 1). The decreased serum FSH concentration on D52 might be – together with reduced serum concentrations of testosterone and oestrogens from D8 to D30 – responsible for a reduced spermatogenesis. A reduction of the quantity of mature spermatozoa was found in the stallion treated in this study (Table 1). The use of a deslorelin acetate implant does not result in infertility and complete loss of libido in stallions (Turkstra, 2005). Histopathological findings confirm the results of the puncture of the tail of the epididymides. A depressed spermatogenetic activity was found in all locations of both testes, which could probably be indicative of a suppressive effect on the germinal epithelium. However, spermatogenesis was retained, albeit being significantly lower. Based on histological data, the stallion could not be considered to be infertile. The use of a GnRH implant leads to a complete reversible infertility in male dogs (Kutzler and Wood, 2005; Trigg et al., 2006; Junaidi et al., 2007). A successful use of the implant in male wildlife animals has also been described (Bertschinger et al., 2001; Kutzler and Wood, 2005).

Until now, little is known about the effects of subcutaneously applied deslorelin acetate implants in stallions. Contradicting findings, i.e. a down-regulation and a stimulating effect on the sexual behaviour on the one hand and
on the sperm quality on the other have been described (Turkstra, 2005). As advantages of an implantation, the reversibility of its effects, good tolerability in many species and the easy handling can be named. One disadvantage is the initially increased stallion-like behaviour demonstrated in this case. It is not clear whether the use of a GnRH agonist to suppress sexual hormones and male behaviour would be regarded to be relevant doping. The use of drugs for medical castration could be considered to be doping or non-allowed medication, because the aim is to influence behaviour and competitiveness. On the other hand, surgical castration seems to have a similar aim, i.e. to reduce the stallion’s male behaviour.

Other methods of medical castration are also not completely without problems. Active immunisation against GnRH causes two forms of immune response, i.e. a lack of response and antibody formation of unpredictable duration. GnRH toxin conjugates have an irreversible effect. Considering these problems, GnRH-agonistic implants could still represent a cheap and user-friendly method to induce a temporary suppression of aggressive behaviour. Due to the individual sensitivity, it could be of interest to test different dosages of GnRH agonists.

In conclusion, the use of a subcutaneously applied deslorelin acetate implant with the aim of changing a stallion’s male behaviour seems to be promising; however, further research is warranted.

Acknowledgements

We thank Laboklin GmbH & Co. KG, Bad Kissingen, Germany and Dr. vét. B. Remy, Reprobiol., Stoumont, Belgium for the analysis of serum samples.

References


