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Effects of implants containing the GnRH agonist deslorelin on testosterone release and semen characteristics in Shetland stallions

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ABSTRACT

The hypothesis in this study was continuous treatment of stallions with the GnRH agonist deslorelin inhibits reproductive functions. A 2-week pre-experimental period was followed by an 11-week deslorelin implant treatment. Stallions received 4.7 (D1, $n = 7$), or 18.8 mg deslorelin (D2, $n = 5$) or remained untreated (C, $n = 5$). Libido, sperm motility, membrane integrity, DNA fragmentation, estrogen receptors, basal plasma testosterone and Anti Muellerian hormone (AMH) concentrations were evaluated once weekly during the treatment period. The testosterone response to the GnRH agonist buserelin and hCG was evaluated twice. In Week 2, stallions in Group C but not Groups D1 and D2 responded to buserelin with testosterone release ($P < 0.001$), while in Week 9, stallions in Group C and D1 but not D2 released testosterone after buserelin administration (group $P < 0.01$, week $P = 0.01$). Stallions of all groups responded to hCG with testosterone release at both times of hCG administration ($P < 0.001$). The AMH concentration was similar in all groups. Deslorelin thus reduced pituitary responsiveness to GnRH but only with a large dose and this effect persisted for several weeks. Total sperm count increased transiently with the D2 treatment but not in stallions of the D1 and C groups after implant insertion (time $P < 0.01$, time x group $P < 0.001$). The percentage of ESR1-positive spermatozoa decreased transiently in Group D2 (time $P < 0.01$, time × group $P < 0.01$). There was no difference among groups at any time during the study in percentage of motile and membrane-intact spermatozoa and sperm with DNA fragmentation. In conclusion, deslorelin implants modulate pituitary function in stallions but not to an extent that affects testicular function.

1. Introduction

Suppression of reproductive functions in stallions can be of interest for population control in feral horses (Garrott and Siniff, 1992), for prevention of male behavior in sport horses (Malmgren et al., 2001) and to eliminate equine arteritis virus in stallions with virus persistence (Stout and Colenbrander, 2004). One approach to suppress reproductive function is blocking the activity of gonadotropin releasing hormone (GnRH) at its pituitary receptors by long-term treatment with GnRH agonists. This results in initial stimulation of LH and FSH release followed by downregulation of pituitary GnRH receptors (Nett et al., 1981; Loumaye and Catt, 1983; Rispoli and Nett, 2005; Smith et al., 2012).

The effectiveness of long-term GnRH treatment to suppress reproductive functions in male animals differs among species. Slow-release implants with the synthetic GnRH agonist, deslorelin, initially have been developed for suppression of fertility and sexual behavior in dogs where these implants reliably inhibit gonadotropin release and spermatogenesis for several months (Junaidi et al.,

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2003, 2009). A complete loss of reproductive function including spermatogenesis has also been reported in boars treated with deslorelin slow-release implants (Kopera et al., 2008, 2009) while in another study, spermatogenesis persisted in one out of five treated boars (Kauffold et al., 2010). In male cheetahs, a consistent loss of spermatogenesis was only obtained with deslorelin doses exceeding that of the commercial 4.7 mg implant (Bertschinger et al., 2006). A more variable response exists in male domestic cats. While in one study, the capacity of Leydig cells to produce testosterone ceased only 4 month after insertion of a 4.7 mg deslorelin implant (Novotny et al., 2012), in another study four of five animals had basal testosterone concentrations at 4 weeks after implant insertion and a GnRH stimulation test did not result in an increase in testosterone concentration (Goericke-Pesch et al., 2013). The decrease in testosterone concentration was associated with reduced male behavior (Novotny et al., 2012). Based on ejaculate characteristics it has been assumed that fertility is maintained for 2–3 months after implant insertion (Novotny et al., 2012; Romagnoli et al., 2017). Also histology of testes revealed the first signs of spermatogenic suppression 2 months after deslorelin implant insertion but at 4 months the amount of the suppression varied among animals (Novotny et al., 2012; Goericke-Pesch et al., 2013; Novotny et al., 2015). In bulls, GnRH receptor downregulation was achieved with slow-release deslorelin implants, but basal LH release, plasma testosterone concentration and spermatogenesis were not suppressed (Aspden et al., 1997, 1998; D'Occhio et al., 1996, 2000).

In stallions, several studies have demonstrated an initial stimulatory effect of GnRH agonist treatment on gonadotropin and testosterone release lasting about 1 week (Boyle et al., 1991; Brinsko et al., 1998; Johnson et al., 2003; Falomo et al., 2013). Thereafter, plasma testosterone concentration decreased in stallions included in most (Boyle et al., 1991; Johnson et al., 2003; Falomo et al., 2013) but not all studies (Brinsko et al., 1998). In three stallions, a decrease in daily sperm production and libido after long-time treatment with the GnRH agonist buserelin has been reported (Boyle et al., 1991). The questions, therefore, remain as to whether spermatogenesis and male sexual behavior can be completely inhibited with long-term GnRH agonist treatment in stallions. In the present study, the hypothesis was tested that slow release deslorelin implants result in an initial stimulation and subsequent downregulation of anterior pituitary and gonadal function in adult stallions, leading to a transient inhibition of spermatogenesis. The research objectives were to determine the effects on testicular functions and sexual behavior. In addition to variables analyzed in part in previous studies on long-term GnRH treatment in stallions, sperm motility, membrane integrity, DNA fragmentation, and estrogen receptor numbers were evaluated to assess sperm function. Furthermore, concentration of Anti-Muellerian hormone (AMH) was analyzed to determine Sertoli cell function and stimulation tests with buserelin and human chorionic gonadotropin (hCG) were performed to assess anterior pituitary and Leydig cell function, respectively.

2. Materials and methods

2.1. Animals

Fertile Shetland pony stallions aged between 6 and 25 years (11.6 ± 1.8 years, mean ± SEM) and weighing between 81 and 217 kg (147 ± 11 kg) were included into the study. Animals were maintained in established groups of four to six stallions in outside paddocks with access to a shed closed on three sides. Stallions were fed hay twice daily and water was available *ad libitum*.

2.2. Experimental design

The study was approved by the Austrian Federal Ministry for Science, Research and Economy (BMWFW-68.205/0073-WF/V/3b/2017) and was conducted in Vienna, Austria (longitude 16.4°, northern latitude 48.3°) starting in April. The experiments consisted of a 2 week pre-experiment period, followed by an 11-week deslorelin implant period. An initial number of 19 stallions were ranked according to age and semen quality and alternately assigned to three groups. Because two stallions died during the study for reasons unrelated to the experiment, only 17 stallions were available until study completion. Stallions received either an implant containing 4.7 mg of the GnRH agonist deslorelin (Suprelorin, Virbac, Vienna, Austria, group D1, $n = 7$), two implants containing each 9.4 mg deslorelin (group D2, $n = 5$) or remained untreated as controls (group C, $n = 5$). Implants in stallions of Groups D1 and D2 were administered subcutaneously on the left side of the horses´ neck.

Collection of ejaculates was performed once weekly throughout the experiment. Blood for analysis of testosterone was taken at weekly intervals from the right or left jugular vein and collected into lithium heparin vacutainer tubes (Becton Dickinson, Schwechat, Austria). Blood samples for testosterone analysis were also taken at 30 and 0 min before and 60 and 120 min after implant insertion (Groups D1 and D2) and respective times in controls (Group C). Blood was centrifuged immediately after collection at 3000 × *g* for 10 min at 4 °C and plasma was stored at −20 °C until analysis.

Stimulation tests with the GnRH agonist buserelin (4 µg i.v.; Receptal, MSD, Vienna, Austria) were performed in weeks 2 and 9 and stimulation tests with hCG (1500 IU i.v.; Chorulon, MSD) were performed in Weeks 3 and 10 after implant insertion (week 1). Blood was collected 30 min and immediately before and 60 and 120 min after buserelin and hCG injections, respectively.

2.3. Semen collection, processing and analysis

Stallions were already acclimated to semen collection before the experiment. Semen was collected with an artificial vagina (Hannover model; Minitube, Tiefenbach, Germany) on a "dummy". For semen collections, stallions were always exposed to an ovariectomized mare expressing estrous behavior for sexual stimulation until erection and readiness to mount, followed by mounting of the "dummy". The time from first presentation to the mare until first mounting of the dummy and the number of mounts until a