Deslorelin subcutaneous implants in *Oryx dammah* males for reproductive control

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Declaration of interest: none

Abstract

The aim of this study was to assess the effects of the deslorelin subcutaneous implant as a temporary contraceptive method in the *Oryx dammah* male. For this purpose, deslorelin at different doses, i.e. 14.1 mg and 9.4 mg, was subcutaneously implanted in three males (Phase 1) and one male (Phase 2) adult *Oryx dammah*, respectively. Quantitative behavior evaluation and androgen concentrations in feces and plasma were assessed before and after implant application. Fecal androgen concentrations observed in treated males were compared with those measured in one orchiectomized male and two females. Fecal androgen concentrations increased up to 15 days after the implant application, then progressively decreased, reaching the basal level at day150 in Phase 1. In Phase 2, levels remained high until day 60 and returned to basal level on day 120. Plasma testosterone concentration was higher on the day of implant application than three months later, but with variable ranges among males. A general increase of activity levels and hierarchical changes were observed after treatment, in accordance with hormonal variations. Despite males cohabiting with two fertile females during the observation period, no births were recorded. However, between the end of Phase 1 and the beginning of Phase 2, i.e. about 10-11 months after the first deslorelin implant, a fertile mating occurred leading to the birth of a calf. Therefore, we can hypothesize a contraceptive effect up to 10 months after the implant. Testicular histology performed on one male at the end of the Phase 2 showed no spermatogenetic activity.

Our results suggest that deslorelin implant can be used to temporarily control reproduction in the *Oryx dammah* male. Behavior and fecal androgen measurements were useful and ~~as~~ repeatable, non-invasive methods to monitor response.

Keywords: deslorelin; implant; *Oryx dammah;* reproduction; testosterone

1. Introduction

The scimitar-horned oryx (*Oryx dammah*) is a seasonal polyestrous species with a variable anestrus period, depending on the climate. This species was once widespread throughout North Africa until being declared extinct in the wild by the International Union for the Conservation of Nature (ww.iucn.org) in 2000, owing to poaching and habitat degradation [1]. Since then, *ex situ* repopulation programs have engaged wildlife parks to conserve this endangered antelope. E*x situ* programs allow an easier control and management of animals beside enabling selection of the most suitable animals for reproduction, avoiding inbreeding and other genetic problems [2,3]. However, at present, uncontrolled reproduction has led to space and resource limitations, and consanguinity risks [4]. In zoos, males seem to be sexually more active in autumn with the births distributed from March to October (www.sandiegozoo.com). However, the sexual behavior, as well as the births, are recorded throughout the whole year in temperate climate [5] such as at Le Cornelle park (Italy) as well as at Safari Park di Varallo Pombia (Italy), making the reproductive control even more difficult. In this context, due to recurrent births within the same small group of animals, the European Association of Zoos and Aquaria (EAZA) decided to regulate reproduction of *Oryx dammah* in wildlife parks.

The main reproductive control methods used in wildlife parks are physical separation of males and females, and surgical and hormonal treatments. Prolonged gender separation can be stressful in gregarious species such as antelopes besides requiring suitable facilities and being perceived as unnatural by park visitors [4]. Surgery represents a definitive option that also implies anesthetic and surgical risks for animals. In the last years, hormonal treatments are becoming popular as reversible or permanent methods of reproduction control and for reducing agonistic behavior [6-9]. Deslorelin is a gonadotropin-releasing hormone (GnRH) agonist used in different species with opposite effects on reproductive function [10-13]. Continuous release of an exogenous GnRH-agonist, such as by subcutaneous implant, can lead to the pituitary down-regulation of GnRH receptor, thus reducing gonadotrophin and reproductive hormone release. As a consequence, gonadal activity is blocked and sexual and aggressive behaviors are reduced [11].

To our knowledge, little information about the use of deslorelin as temporary contraceptive method

in *Oryx dammah* is available. Serum testosterone has been reported to increase two mo after 2 X 6 mg deslorelin implant treatment while aggressive behavior did not appear to change in a previous study involving two males [14]. Endocrine measurement is the most precise of the indirect methods for monitoring the reproductive function [15 ~~14~~], but physical restrain and anesthesia are required to collect blood samples from wild animals, such as *Oryx dammah*. Therefore, non-invasive methods such as fecal hormonal analysis are preferred to assess reproductive status in wild animals [16]. The aim of the present study was to examine the contraceptive efficacy and duration of deslorelin subcutaneous implants in *Oryx dammah* by monitoring the animals for an extended period using minimally invasive procedures, i.e. fecal hormonal analysis and behavioral observations.
We hypothesized that deslorelin administration in males would mitigate reproductive and aggressive behaviors, and reduce fecal androgen concentrations to levels comparable to those observed in neutered male and female *Oryx dammah*.

2. Materials and methods

This study was carried out at *Le Cornelle* park, in Northern Italy (45°42′ N 9°35′ E), between April 2016 and December 2017. Neutering was required by the EAZA coordinator as a routine treatment for population management.

2.1. Animals

The herd was composed of three adult males receiving deslorelin treatment (M1, M2, M3; Table 1) plus one orchiectomized male (OM: 5-y old) and two females (F1: 4-y old; F2: 1-y old), which served as control group (CTR) in studying fecal androgen concentrations (Table 1). The remote reproductive history of the intact animals involved in the study reported no mating in M1 and F2, while both M2 and M3 mated with F1 produced offspring 10 mo and just before starting the Phase 1, respectively.
All animals were housed together in an outdoor pen (40 X 50 m) with sand, gravel, grass and rock areas, trees and an indoor shelter. They were fed with *ad libitum* hay supplemented with mixed cereals, fruit and vegetables. Water was provided *ad libitum*. All animal-related procedures were approved by the EAZA.

2.2. Treatments

The study lasted 20 mo and consisted of two phases. In Phase 1 (from April 2016 to January 2017), the three adult males (M1, M2, M3) were treated with 14.1 mg deslorelin dose. In all males, behavioral observations and fecal collections were performed until 90 days and 150 days after

implant, respectively. In Phase 2 (from March 2017 to December 2017), the treatment was repeated with 9.4 mg deslorelin dose only in one male (M1), because M2 and M3 died before the beginning of Phase 2 (February 2017), due to causes not related to the deslorelin implant. A detailed description of treatments in these two phases is provided below.

- *Phase 1:* In June 2016, M1, M2, M3 were each treated with three 4.7 mg deslorelin subcutaneous implants (Suprelorin, Virbac, Milano, Italy), a total dose of 14.1 mg. Implants were inserted subcutaneously over the shoulder after animals underwent sedation using detomidine (0.03 mg/head im, Domosedan, Orion Corporation, Turku, Finland), ketamine (1.2 mg/head im, Ketavet 100, Intervet Productions, Aprilia, Italy) and xylazine (0.2 mg/kg im, Rompun, Bayer, Leverkusen, Germany).

- *Phase 2:* In May 2017, 11 mo after the first treatment, M1 was treated again with two 4.7 mg deslorelin implants, at a total dose of 9.4 mg, as described for Phase 1.

2.3. Behavioral observations

In Phase 1, direct behavioral observations were carried out weekly on treated males, starting from 36 days pre-treatment until 92 days after treatment, for a total of 20 observation days. Each observation period lasted for one hour, from 07:00 a.m. to 08:00 a.m., during which each male was observed randomly for 20 min. Observations were performed using a continuous recording and focal animal sampling technique [17], from a fixed point with a complete view of the paddock.

The ethogram included the following general categories of maintenance behaviors: moving (walking or running, head upright); feeding (eating hay, cereals or vegetables); ruminating (performing rumination, either standing or lying); standing (quadrupedal posture); lying inactive (body on ground, head up or down, no visible sign of activity); alert (standing, lying or walking with head raised and ears positioned forward); self-grooming (licking parts of own body); exploration (licking the environment or sniffing around). Additionally, specific behaviors related to reproductive activity were recorded: courtship (moving around the female, following the female, sniffing or licking her perianal region, resting with the chin or placing the foreleg on the female's body, flehmen, mounting, with or without ejaculation); marking (scratching the ground, urine spraying, defecation on or close to the feces of other males); agonistic_M (threats and aggressions directed at other males); agonistic_F (threats and aggressions directed toward females).

Behaviors like vocalizations, allo-grooming, defecation, urination and playing that occurred with very low frequencies were grouped together into the "other" category and were not considered for further analysis.

137 *2.4. Collection of fecal and plasma samples*

138 Fecal and plasma samples were collected at various time intervals and frequencies, according to the
139 opportunities offered by the daily management of the zoological park. To collect fecal samples, each
140 animal was confined overnight in one of the shelters within the enclosure, paying specific attention
141 to clean the shelter floor to avoid sample contamination. In the morning, fecal samples (approximately
142 10 g) were collected from the shelter floor and stored in sealed plastic bags at -20°C until analysis.
143 In animals intended to receive the deslorelin treatment (M1, M2, M3), collection of fecal samples
144 began ~ two mo before implantation (Pre) in both phases. Fecal samples were collected on: days 2-7
145 (D7), days 8-15 (D15), days 16-30 (D30), days 31-60 (D60), days 61-90 (D90), days 91-120 (D120)
146 and days 121-150 (D150). During Phase 1, the frequency of the fecal sample collection was more
147 intense in the first month after implant insertion, and decreased in the following months. During Phase
148 2, a lower number of fecal samples could be collected during the first two wks after implant insertion
149 due to management reasons. Several fecal samples were also collected from CTR animals throughout
150 both experimental phases.
151 Plasma testosterone was measured in plasma samples obtained from M1, M2, M3 during medical
152 examinations. Biochemical examination and blood count were also performed in both phases at the
153 implant insertion and three mo later as required by the EAZA.
154 At the time of deslorelin implantation in the treated group, plasma testosterone was measured once
155 also in CTR animals. Blood samples (5 mL) were collected from the jugular vein in vacuum K_2EDTA
156 tubes, centrifuged (3500 X g, 20 min, room temperature). The plasma obtained was aliquoted into 1
157 mL Eppendorf vials and stored at -20°C until analysis.
158
159 *2.5. Hormone solvent extraction*

160 Before analysis, each fecal sample was thoroughly mixed. To extract steroid hormones from the fecal
161 matrix, 200 mg of the wet samples were double-extracted by boiling in 90% ethanol as described by
162 Vernocchi et al. [18]. The ethanolic extracts were dried under nitrogen flow and re-dissolved in 0.25
163 mL PBS.
164 Plasma samples (0.1 mL) were extracted with 8 mL of diethyl ether by mixing for 30 min on a rotary
165 mixer. The tubes were centrifuged at 2000 X g for 15 min, the supernatants were transferred into fresh
166 glass tubes and dried under nitrogen flow at 37 °C. The dried extracts were dissolved in 1 mL PBS
167 containing 0.1% BSA (pH 7.4) and were shaken for 10 min [19].
168
169 *2.6. Androgen radioimmunoassay*

170 Androgen concentrations were measured in fecal (0.05 mL) and plasma (0.1 mL) extracts by a solid-

phase microtiter radioimmunoassay (RIA) [19]. The specific antiserum was raised in a rabbit against testosterone-3,carboxymethyloxime-BSA and showed the following tested cross-reactions: testosterone 100%, 5α-dihydrotestosterone 38%, 5α-androstan-3α,17β–diol 13.7%, 5α-androstan-3β,17β-diol 13.7%, 19-nortestosterone 8.6%, androstenedione 1.6%, 5-androstene-3β,17β-diol 1.2%, DHEA 0.01% and ≤ 0.01% for estradiol, progesterone and cortisol. The tracer was 1,2,6,7-^{3}H testosterone (Perkin-Elmer Life Science, Shelton, CT) and the calibration curve was constructed using native testosterone (Sigma-Aldrich, St. Louis, MO) as the standard. The detection limit of the assays was 3.1 pg/well. The repeatability was expressed as intra and inter-assay coefficients of variation, which were 4.6% and 7.7%, respectively. Although the assay is specifically designed to measure testosterone, data obtained from fecal samples are expressed as androgen concentrations, as it cannot be excluded that unknown metabolites of androgen or other steroids different from native testosterone are present in oryx feces and cross-reacted with the antiserum.

2.7. Histology

At the end of Phase 2 (December 2017), the M1 male was orchiectomized due to the onset of a sexually transmitted disease within the group. Both gonads were sent to the laboratory for histological analysis. Fragments of each testis were collected and immediately immersed in formalin 10% for 24–48 h at 4°C. After fixation, fragments were dehydrated in a graded series of ethanol, clarified in xylene and embedded in paraffin. Serial sections were cut at 4-μm thickness, dewaxed and stained with hematoxylin and eosin for general morphological purposes.

2.8. Statistical analysis

Behavioral data were expressed as the percentage of time spent performing each behavioral category out of the total observation time. Behavioral trends were graphically represented using the same time intervals as for fecal androgen samples (Pre, D7, D15, D30, D60, D90). Differences between pre- and post-treatment for all behavioral categories and differences between subjects for reproduction-related behaviors were compared by non-parametric analysis of variance (Mann-Whitney test).

The Kruskal-Wallis non-parametric one-way ANOVA and multiple post-hoc comparisons were used to compare fecal androgen concentrations among the different time intervals (Pre, D7, D15, D30, D60, D90, D120, D150) from implant insertion and CTR samples. Both behavioral and hormonal data were analyzed using IBM SPSS Statistics, Version 25.

3. Results

205 No births were recorded during the observation periods, i.e. 10 mo (Phase 1) and 20 mo (Phase 2)
206 after the first deslorelin treatment. However, at the beginning of Phase 2 and before the second
207 deslorelin treatment, a fertile mating occurred between M3 and F1, leading to the birth of a calf on
208 February 2017.
209
210 *3.1. Phase 1*
211 The percentage of time dedicated to maintenance behaviors pre- and post-treatment out of the total
212 observation time is presented in Table 2, and their temporal trend is shown in Figure 1. After the
213 implant insertion, a general intensification of the level of activity occurred: standing and alert
214 increased ($P < 0.05$; Table 2) and a slight but significant increase of exploration, with a peak at D15
215 (Fig. 1), was observed ($P < 0.05$; Table 2). The increase of moving approached statistical significance
216 ($P = 0.06$; Table 2), with a peak at D60, which corresponds also to the peak of standing and to the
217 lowest percentage of time spent feeding (Fig. 1). For behaviors related to reproductive activity
218 (marking, courtship, agonistic_M, agonistic_F), a more detailed individual analysis of behavioral
219 trends was carried out and is presented in Fig. 2.
220 Individual differences before and after treatment were not statistically significant. The overall time
221 dedicated to courtship differed among males (M1 = 0.38 ± 0.16; M2 = 2.02 ± 1.08; M3 = 13,71 ±
222 2.72% of time; $P < 0.001$). In particular, in the first month after treatment this behavior showed a
223 marked decrease in the originally dominant and more sexually active male M3, and a correspondent
224 increase in the originally medium ranking male M2. However, at D60 M3 increased courtship again,
225 while M2 went down to the pre-treatment level. These behavioral changes were accompanied by a
226 peak of marking behavior at D15 in M2 and by an initial increase of the agonistic behavior *vs* other
227 males in M3, that then decreased at D60. The percentage of time dedicated by M1 to agonistic
228 behavior *vs* males was significantly lower than in the other two males ($P < 0.001$). The agonistic
229 behavior *vs* females did not differ among subjects.
230 Fecal androgens concentrations observed during Phase 1 are shown in Figure 3 A.
231 Mean fecal androgen concentrations were 12 ± 4 ng/g (mean ± SD) in CTR samples and 33 ± 15 ng/g
232 in the treated animals (M1, M2, M3) before implant insertion ($P < 0.05$). The higher fecal androgen
233 concentrations (52 ± 35 ng/g) were observed on D7 ($P < 0.05$). From D15 to the end of the
234 experimental period, fecal androgen concentrations were comparable to those observed in CTR
235 animals and in treated animals before deslorelin administration. On average, M3 showed the highest
236 fecal androgen concentrations until D60.
237 Plasma testosterone measured on the day of implant insertion was lower in M1 (0.6 ng/mL) than in
238 M2 (6.2 ng/mL) and M3 (6.0 ng/mL). Three months after implant insertion, plasma testosterone was

239 0.5 ng/mL in M1, 2.6 ng/mL in M2 and 5.6 ng/mL in M3. Basal levels of plasma testosterone
240 measured on the same day of implantation in CTR animals ranged from 0.6 to 1.2 ng/mL.
241
242 *3.2. Phase 2*
243 Fecal androgens concentrations observed during Phase 2 in the M1 *Oryx dammah* male receiving a
244 second deslorelin treatment are shown in Figure 3 B. Mean fecal androgen concentrations were 14 ±
245 5 ng/g in CTR samples and 19 ± 2 ng/g in M1, before implant insertion. Higher concentrations ($P < 0.05$) were observed on D15. Fecal androgen concentrations in M1 were comparable to those
247 observed in CTR samples from D90.
248 Plasma testosterone concentration was 2 ng/mL at the deslorelin implantation time in M1 and it
249 decreased to 0.6 ng/mL three months later.
250 At the end of Phase 2 (200 days after the second deslorelin implant insertion), M1 underwent
251 orchiectomy. Testicular histology showed an inactive gonad. The testicular parenchyma and *rete*
252 *testis* showed a well-developed aspect. The seminiferous epithelium lining the seminiferous tubules
253 was made up of cells related to early phases of the spermatogenesis, which is spermatogonia and
254 primary spermatocytes. Normal vascularization and a moderate number of Leydig cells were present
255 in the interstitial spaces. Very few spermatids and even fewer sperms were sporadically detected in
256 some sections of the seminiferous tubules in both testes (Figure 4).
257
258 **4. Discussion**
259 Cohabitation of surplus captive antelopes can result in serious male aggression, leading to injury or
260 death, besides increasing overpopulation in zoos [14]. Testosterone plays a critical role in
261 modulating male sexual development and activity [20]. Thus, by reducing testosterone
262 concentrations, neutering may have a double control function both on aggressive interactions and
263 on unwanted births [14,21]. A reversible system of reproduction control seems to be more
264 appropriate than a permanent surgical approach in endangered species, such as *Oryx dammah*.
265 Furthermore, the former approach avoids post-operative pain and complications that are particularly
266 difficult to manage in wild animals.
267 Our results confirmed the initial hypotheses of a deslorelin effect on behavior and reproduction in
268 *Oryx dammah* males and of the diagnostic role of fecal androgen measurements. Indeed, a
269 temporary change in the hierarchy occurred immediately after implant and modified the original
270 linear hierarchy among males (M3 dominant over M2; M2 dominant over M1), which can usually
271 be found in wild oryx herds [22]. In particular, when androgen levels decreased in M3, the animal
272 lost its dominant role and decreased courtship behavior whereas M2, increased courtship and

marking behavior, becoming the dominant male. Behavioral observations confirmed that M1 was the less sexually active male, and its courtship behavior was almost absent throughout the study. In Phase 2, when M1 became dominant subject in the herd over the neutered male and the females (as commonly observed in the wild)[22], its androgens rose until D60.

The acute response to the GnRH agonist deslorelin involves the activation of GnRH receptors leading to hyper-secretion of FSH and LH, and then of reproductive steroid hormones (flare effect). The absence of births occurring during the study period together with low fecal hormone levels in treated animals suggest an impairment of reproductive functions due to deslorein implant. After an initial stimulatory effect, continued doses of deslorelin (chronic response) result in a down-regulation of its receptors in the pituitary gland and a shutdown of FSH and LH release which subsequently suppresses reproductive function [13]. In agreement with the literature [14,23], an increase of fecal androgen concentration up to D15 after deslorelin implantation was recorded in both phases of our study. This transitory increase of androgen level is probably responsible for the behavioral changes observed in response to treatment, with a general increase of activity levels, as recorded also by Penfold et al. [14]. The fecal androgens subsequent trend varied depending on deslorelin dose, i.e. progressively decreased until reaching basal values at D150 (Phase 1), while it remained high until D60 and reached basal level on D120 (Phase 2) after treatment. A similar negative trend was reported in deer [24] and dogs [25] with different timing. However, individual differences were observed among the three *Oryx dammah* males. In agreement with behavioral data, showing that M3 was initially the most dominant and sexually active male, M3 had the highest pre-treatment fecal androgen concentrations, which remained higher than in the other two males (M1 and M2) up to D60 after implantation. During Phase 1, the youngest subordinate male (M1) showed the lowest androgen concentrations both pre- and post- deslorelin implant. Reversible effects of the implant were supported by the fertile mating of M3 that occurred at the beginning of Phase 2 and before the second deslorelin implant (that is about 11 months after the first implant), which led to the birth of a calf in February 2017. Moreover, at the beginning of Phase 2, i.e. eleven months after the first deslorelin implant, fecal androgens were higher in M1 than both in control ones and in M1 in Phase 1 pre-treatment. Furthermore, 90 days after the first deslorelin implant, we observed a general trend to increase feeding, moving, and standing and, in M1, marking behaviors also increased compared to pre-treatment levels in M1. These findings are suggestive of gonadal activity recovery.

The measurement of plasma gonadal steroid levels is considered the most accurate method to evaluate hormonal secretion [15,26]. However, endocrine monitoring by blood collection is a challenging task in species like the oryx, which require physical restraint for such procedures [27].

It should be noted that anesthetic drugs, such as detomidine, ketamine and xylazine used in our protocol, might lead to a transient alteration of testosterone in laboratory animals [28]. The same response may occur in *Oryx dammah* males, thus explaining the high testosterone levels recorded at D90 after deslorelin implant. Moreover, plasma testosterone levels can fluctuate over time due to a multiplicity of factors, including social dominance as described in bulls, rams and rhesus monkeys, showing greater variation than fecal samples [20,29-31]. The rapid oscillatory pattern in blood testosterone levels suggests the need of cautious interpretation of the meaning of a single value [32]. Thus, whilst fecal samples represent a cumulative measure of hormones over time and allow multiple repeated collection with minimal disturbance to the animal, plasma provides a 'snapshot' that reflects immediate responses to environmental events [20,33-35]. Our data confirmed fecal sampling as a more suitable method than blood collection to assess hormonal concentration and gonadal activity throughout the long observation period. In fact, plasma testosterone levels did not provide a clear indication of the block of reproductive function, whereas our data on fecal androgen concentration, together with the cessation of births, suggest that deslorelin has an effective contraceptive effect. A double blood testosterone measurement performed only once before and once after deslorelin implantation did not diagnose the induced GnRH pituitary down-regulation as also recorded by Penfold et al. [14]. Conversely, fecal androgens together with quantitative behavior evaluation were useful in detecting responses to treatment.

Based on fecal androgen levels, we maintain that deslorelin implant effectively acts as contraceptive method for at least ten mo. In fact, M1 fecal androgen values at the second deslorelin treatment were as low as those preceding the first implantation. Moreover, no births were recorded through the 20-month observation period. Spermatogenesis inhibition was also confirmed by testes histology. Histological examination of hematoxylin and eosin-stained testicular tissues was aimed at verifying the spermatogenetic activity after deslorelin subcutaneous implant as a temporary contraceptive method. Both testes of M1 male orchiectomized at the end of Phase 2 showed a quite silent aspect. The presence of a very small amount of spermatids in rare seminiferous tubules and of very few spermatozoa at epididymal level might conceivably be related to initial recovery of spermatogenic activity seven mo after the hormonal treatment.

5. Conclusion

Long-acting deslorelin may be a practical alternative to neutering to suppress testes function in the *Oryx dammah* male. Deslorelin implants allowed controlling aggressive and sexual behavior together with reducing fecal androgen concentrations. Measurement of androgens in feces is a non-invasive, repeatable, accurate method for reproductive monitoring.

Further studies should be performed in *Oryx dammah* males focused on optimizing deslorelin dose needed to temporarily control reproduction and to identify the total period of effectiveness.

Conflicts of interest

The authors have no conflict of interest in publication of this research article.

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Figure Captions

Table 1.

Age and characteristics of the three *Oryx dammah males* enrolled in the present study.

Table 2.

Percentage of time (means ± SE) dedicated to maintenance behaviors before (pre-treatment) and after (post-treatment) the subcutaneous implant of deslorelin during Phase 1 from all treated animals.

* indicates $P < 0.05$; Mann-Whitney test.

Fig. 1.

Temporal trend of maintenance behaviors (percentage of time out of the total observation time) in relation to the time intervals from deslorelin implant insertion during Phase 1 (mean percentage of time ± SD) from all treated animals.

Pre means before deslorelin implant; D7 means observation between day 2 and day 7; D15 means observation between day 8 and day 15; D30 means observation between day 16 and day 30; D60 means observation between day 31 and day 60; D90 means observation between day 61 and day 90.

Fig. 2.

Temporal trend of individual reproductive behaviors (percentage of time out of the total observation time) in relation to the time intervals from deslorelin implant insertion during Phase 1 (mean percentage of time ± SD) from all treated animals.

Agonistic_M means threats and aggressions directed at other males; Agonistic_F means threats and aggressions directed toward females; Pre means before deslorelin implant; D7 means observation between day 2 and day 7; D15 means observation between day 8 and day 15; D30 means observation between day 16 and day 30; D60 means observation between day 31 and day 60; D90 means observation between day 61 and day 90.

Figure 3.

Comparison between fecal androgen concentrations measured in the three *Oryx dammah* males receiving deslorelin implants (M1, M2, M3) and in the control animals (CTR; one orchiectomized male and two females) during phases 1 (graph A) and 2 (graph B) of the experiment.

CTR means control animals; fecal samples were collected before the insertion of deslorelin implant (Pre) and during the following time intervals: days 2-7 (D7), days 8-15 (D15), days 16-30 (D30),

days 31-60 (D60), days 61-90 (D90), days 91-120 (D120) and days 121-150 (D150). The black horizontal bars represent the medians of the fecal androgen concentrations observed in each time interval. Different superscripts ($^{a, b, c}$) in the same graph indicates significantly different distributions of androgen concentrations ($P < 0.05$; Kruskal-Wallis ANOVA).

Figure 4.

Sections of the testis of M1 orchiectomized male at the end of Phase 2.

Only early stages of the spermatogenesis can be seen in the seminiferous epithelium lining the seminiferous tubules: spermatogonia lie on the basal lamina and primary spermatocytes are highlighted by asterisks (*). Arrows indicate Leydig cells in the interstitial spaces. Hematoxylin and eosin stain. Original magnification X 40.

478 Table 1.

Id.	Age (y)	Characteristics
M1	3	low-ranking male; small body size
M2	10	medium-ranking male; medium body size
M3	9	highest-ranking male; robust constitution

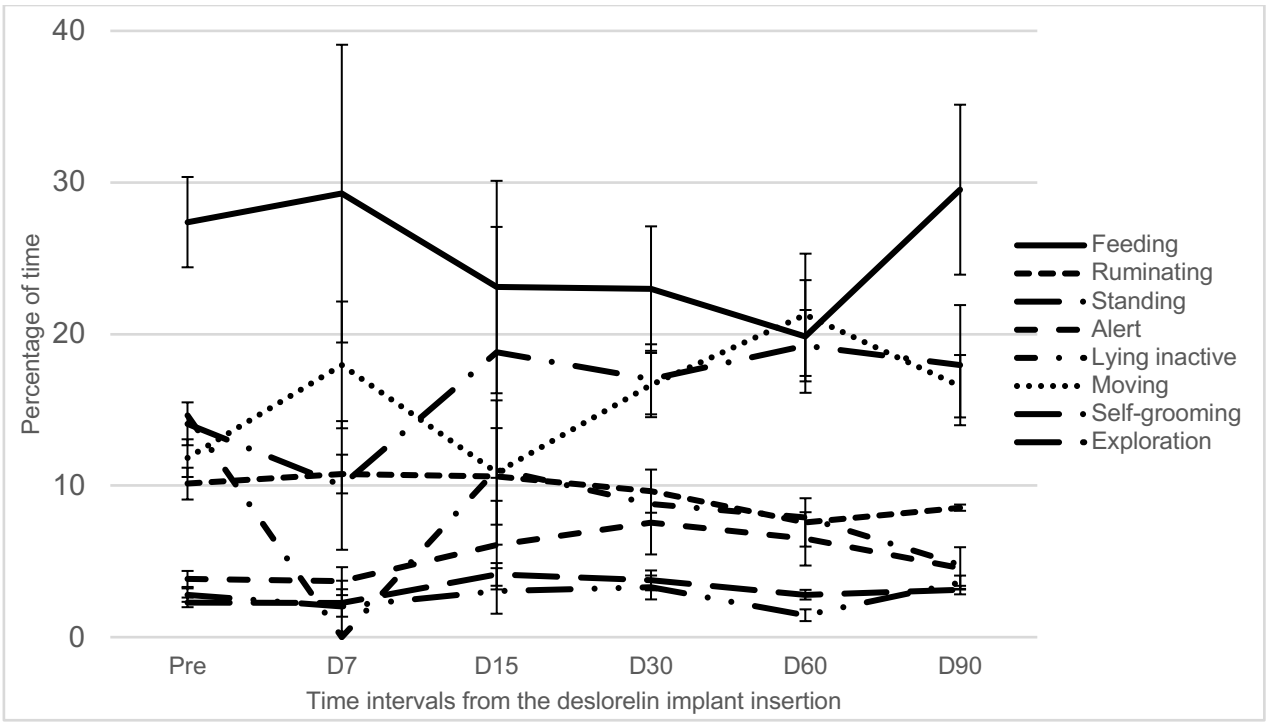
479

480 Table 2.

Behavioral category	Pre-treatment		Post-treatment		P
	mean	SE	mean	SE	
Feeding	27.38	2.98	23.87	1.31	0.124
Ruminating	10.13	1.05	10.64	0.46	0.938
Standing	14.08	1.41	15.48	1.11	0.029*
Alert	3.84	0.53	3.42	0.48	0.047*
Lying inactive	14.63	3.81	15.93	2.60	0.645
Moving	11.81	1.24	13.44	1.17	0.060
Self-grooming	2.78	0.44	3.54	0.26	0.979
Exploration	2.30	0.32	2.28	0.17	0.030*

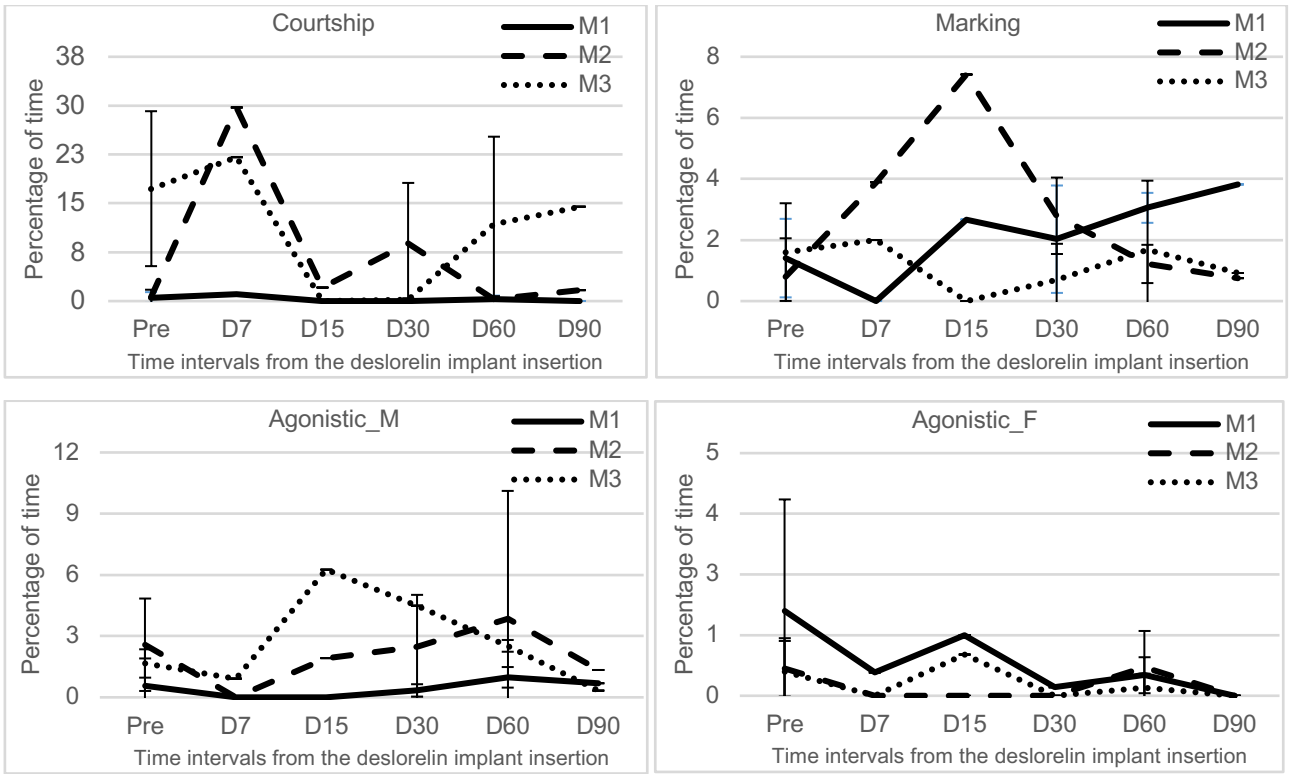
481

482 Fig. 1.


40
30
20
10
0
Percentage of time
Pre
D7
D15
D30
D60
D90
Time intervals from the deslorelin implant insertion
Feeding
Ruminating
Standing
Alert
Lying inactive
Moving
Self-grooming
Exploration

483

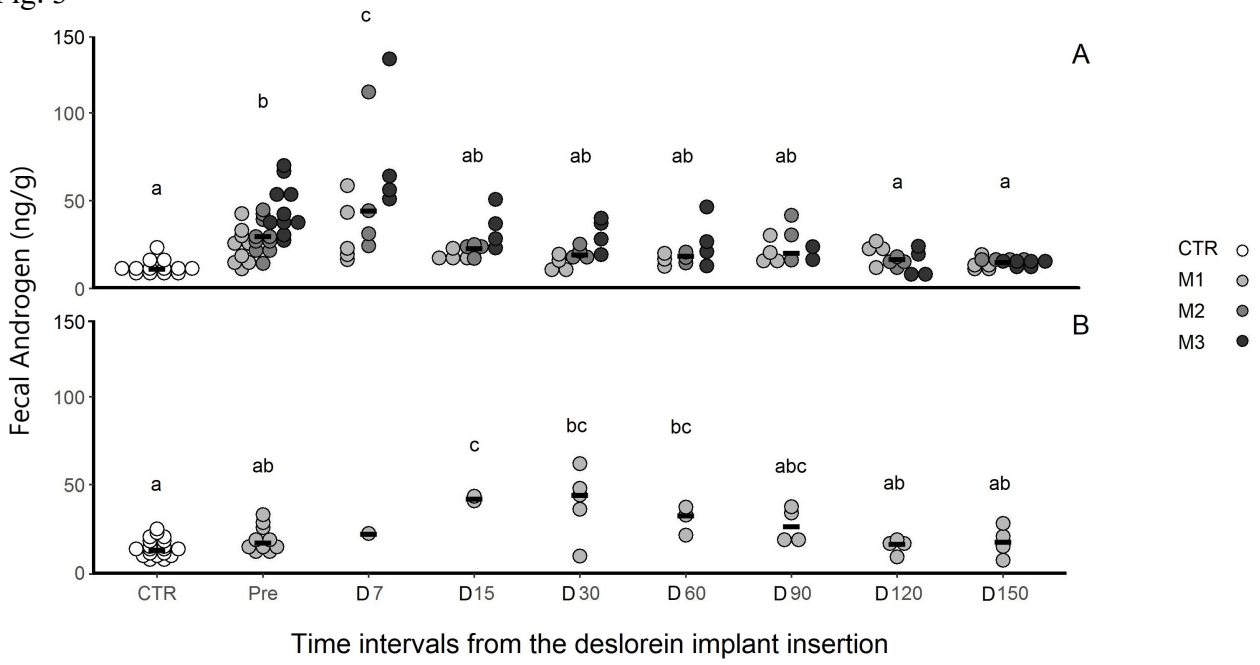
484 Fig. 2.



485

486

487 Fig. 3


A
B
Fecal Androgen (ng/g)
150
100
50
0
a
b
c
ab
ab
ab
ab
a
a
a
ab
c
bc
bc
abc
ab
ab
CTR
Pre
D7
D15
D30
D60
D90
D120
D150
Time intervals from the deslorein implant insertion
CTR
M1
M2
M3

488

Fig. 4