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COMMUNICATION

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COMMUNICATION

Suppression of ovarian activity in a captive African Lion *Panthera leo* after deslorelin treatment

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Abstract: With the intent to evaluate the efficiency of a contraceptive treatment for cyclic ovarian suppression in African Lionesses *Panthera leo* using a Gonadotrophin-Releasing Hormone (GnRH) agonist bioimplant, noninvasive fecal steroid assay associated with the observation of the behavioral estrus were employed for a period of 36 months. Five captive adult females, maintained with a vasectomized male, subcutaneously received a 9.4mg deslorelin acetate implant. The treatment initially stimulated behavioral estrus along with ovarian activity, demonstrated by an estrogen increase in two lionesses. A rise in progesterone concentration in two other animals suggested possible treatment-induced ovulation. After the initial period, deslorelin prevented ovarian activity for at least 22 months. Two females exhibited signs of behavioral estrus after 22 and 31 months. A third lioness with an increased estrogen concentration did not exhibit behavioral estrus signs or a consequent progesterone surge until 33 months after implantation, suggesting a possible resumption of ovarian activity. One female did not exhibit any behavioral estrus signs nor a rise in steroid levels after the "treatment-induced" estrus throughout the entire experiment (36 months). One lioness died after 15 months without exhibiting signs of estrus or an increased progesterone level, however, the estrogen concentration increased 12 months post-implantation, suggesting resumed ovarian activity. The study showed that long-term treatment with a GnRH agonist can be extremely effective as a contraceptive treatment in African lionesses, however, the duration of contraception may vary among individuals and may bear the risk of permanent loss of normal ovarian activity.

Keywords: African Lion, contraception, estrus behavior, fecal assay, GnRH agonist.

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Portuguese abstract and **Author contribution** see end of this article.

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INTRODUCTION

The reproduction of wild animals in captivity is an important tool for ex situ conservation of endangered species (Jorge Neto et al. 2018b). Some species such as the African Lion *Panthera leo*, however, can adapt to captivity, and thus, are capable of reproducing in such an environment. The abundant reproduction of large carnivores is associated with low adult mortality and increased longevity in captivity. This creates a number of complications as the physical space and financial resources available for their maintenance is limited (Woodroffe & Frank 2005).

The objective of the present study was to use the noninvasive fecal steroid assay associated with behavioral estrus to evaluate the efficiency of chronic treatments with the Gonadotrophin-Releasing Hormone (GnRH) agonist bioimplants to suppress cyclic ovarian activity in African Lionesses.

MATERIALS AND METHODS

Experimental Design

Five adult African Lionesses (L1, L2, L3, L4 and L5) were maintained in captivity with a vasectomized male at the Zoological Park of São Paulo. All females had at least one confirmed pregnancy with a live birth, and none of them had been previously submitted to any kind of contraceptive management, except for physical separation from male lions and time with vasectomized males. L1 (13 years old), L2 (6 y/o) and L4 (6 y/o) were born in the São Paulo Zoo, while L3 (7 y/o) and L5 (7 y/o) came from another captive facility when they were six months old.

The five lionesses received a 9.4mg deslorelin acetate implant subcutaneously. The efficiency of the implant as a contraceptive was evaluated non-invasively using a fecal steroid assay and through observation of the behavioral estrus. The study was approved by the University's Ethics Committee for Use of Animals in Research (CEUAVET-USP).

Gonadotrophin-Releasing Hormone Agonist Bioimplant Formulation and Implantation

The GnRH agonist bioimplants used in the present experiments were supplied by Peptech Animal Health Pty Limited, Australia (Suprelorin 9.4 mg; No. 978; Batch DR023). Each implant contained 9.4mg of GnRH agonist deslorelin acetate ($C_{64}H_{83}N_{17}O_{12}$). Implants were placed subcutaneously under aseptic conditions using a commercial implanting device.

Sample Collection, Hormone Extraction, and Dosage

During the experiment, two fecal samples were collected twice weekly, sealed in plastic bags, labeled with the individual's name/date, and stored at -20°C. From 45 days before to 36 months after implant, fecal aliquots were extracted to quantify estrogen and progestogen metabolites. Fecal hormone metabolites were extracted from the samples, as previously described (Brown et al. 1994). Briefly, each fecal sample was lyophilized, pulverized, and 0.18–0.2 g of dry fecal powder was boiled in 5mL of 90% ethanol for 20min. During boiling, 100% ethanol was added as needed, to maintain approximate pre-boil volumes.

After centrifugation (500g, 20min.), the supernatant was recovered, and the pellet re-suspended in 5mL of 90% ethanol, vortexed for 30 sec, and re-centrifuged (500g, 15min.). The first and second supernatants were combined, air dried, and reconstituted in 1mL methanol. Methanol extracts were vortexed briefly and placed in a sonicator for 15min. Each extract was diluted 1:10 in a steroid dilution buffer and stored in polypropylene tubes at -20°C until further use.

Subsequently, each sample extract was assayed for estradiol and progesterone metabolites following RIA. Estradiol Coat-a-Count RIA kits (Diagnostic Products, Los Angeles, CA, USA) were used to measure the estradiol metabolites, while Progesterone DSL-3900® RIA kits (Diagnostic System Laboratories Inc., Webster, USA) were used to measure the progesterone metabolites. Samples were analyzed in duplicate, and those with a coefficient variation of more than 15% were either re-analyzed (if there was enough sample volume for re-analysis) or discarded.

Estrus Behavior Observation

Animals were observed for 30 min periods twice each day (during the morning and the afternoon), three times a week. The following estrus behavioral patterns were recorded (Schaller 1972): vocalization, restlessness, increased frequency and intensity of rolling, lordosis, male attraction, mating acceptance, and copulation.

RESULTS

Before implant placements, all animals had normal ovarian activity, as confirmed by fecal hormone metabolites dosages (figs. 1–5) and behavioral estrus signs, such as vocalization, restlessness, increased

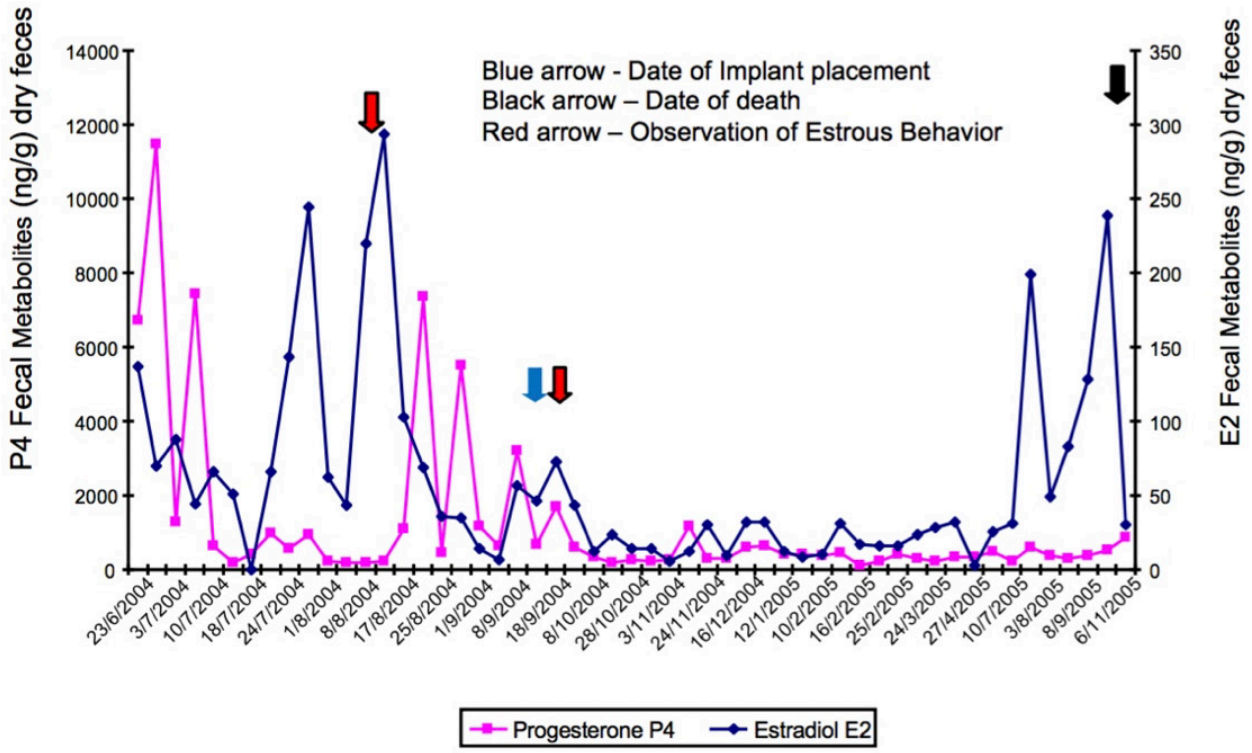

Figure 1. Fecal steroid metabolites profile of the lioness L1 (Panthera leo). Blue arrow—date of Implant placement | Black arrow—date of death | Red arrow—observation of estrous behavior.

Table 1. Rise in fecal steroids concentration and/or estrus behavior shortly after implant placements, and period of contraception in African lions treated long-term with GnRH agonist (deslorelin).

Lioness	Estrus behavior shortly after implantation	Rise in fecal progesterone shortly after implantation	Rise in fecal estrogen after downregulation	Estrus behavior after downregulation	Rise in fecal progesterone after downregulation
L1	Yes	No	12 months	Not observed*	Not observed *
L2	No	Yes	No	22 months	Not observed**
L3	Yes	No	No	31 months	Not observed**
L4	No	No	No	33 months	33 months
L5	No	Yes	No	Not observed**	Not observed**

frequency and intensity of rolling, lordosis, male attraction, mating acceptance, and copulation. The average estrus length was 5.8 ± 2.2 days. Treatment with deslorelin initially stimulated a behavioral estrus along with ovarian activity, as demonstrated by increases in the estrogen concentration in two lionesses (L1 and L3, Figs. 1 and 3). We also noted a rise in progesterone concentration in two other females (L2 and L5, Figs. 2 and 5), which suggests possible treatment-induced ovulation (Table 1). After this period, the GnRH agonist prevented ovarian activity for at least 22 months.

Two lionesses exhibited behavioral estrus signs 22 and 31 months after implantation, respectively (L2 and L3, Figs. 2 and 3, respectively). In a third lioness (L4, Fig. 4), behavioral estrus signs and increases in estrogen concentration, as well as a consequent surge in progesterone level was noted 33 months after implant use. The lioness L5 (Fig. 5) did not exhibit any signs of behavioral estrus. Moreover, she only experienced a rise in female sex steroids levels (estrogen and progesterone) after the "treatment-induced" estrus the end of the experiment (36 months). The lioness L1 (Fig. 1) died 15 months after experiment initiation, without demonstrating any estrus signs, nor a rise in progesterone level, however, her estrogen concentration increased 12 months after the placed implant (Table 1).

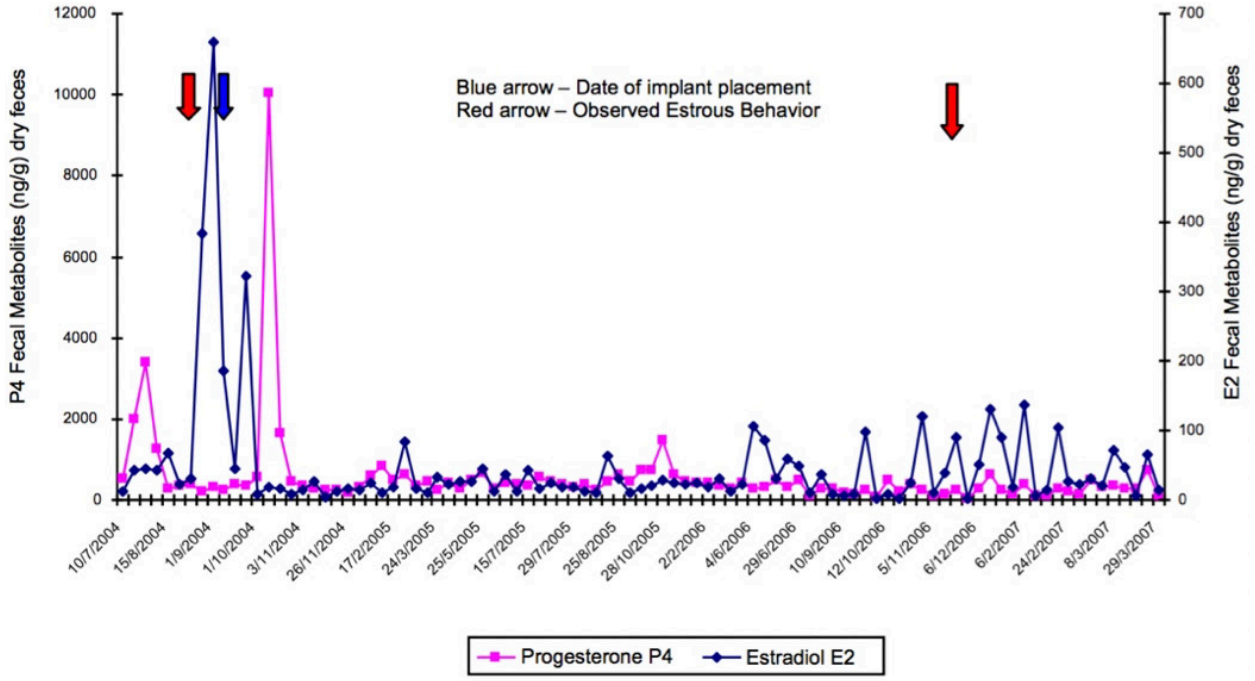

Figure 2. Fecal steroid metabolites profile of the lioness L2 (Panthera leo). Blue arrow—date of implant placement | red arrow—observed estrous behavior.

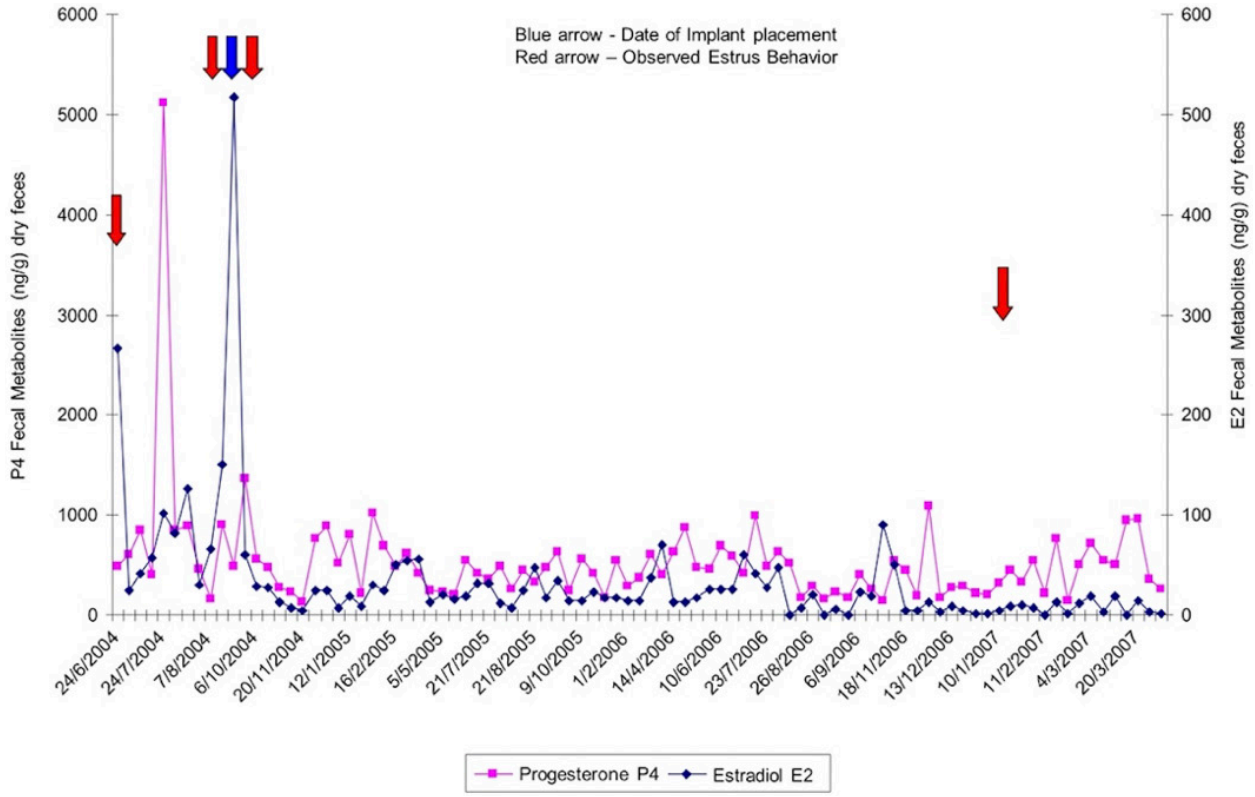

Figure 3. Fecal steroid metabolites profile of the lioness L3 (Panthera leo). Blue arrow—date of implant placement | red arrow—observed estrous behavior.

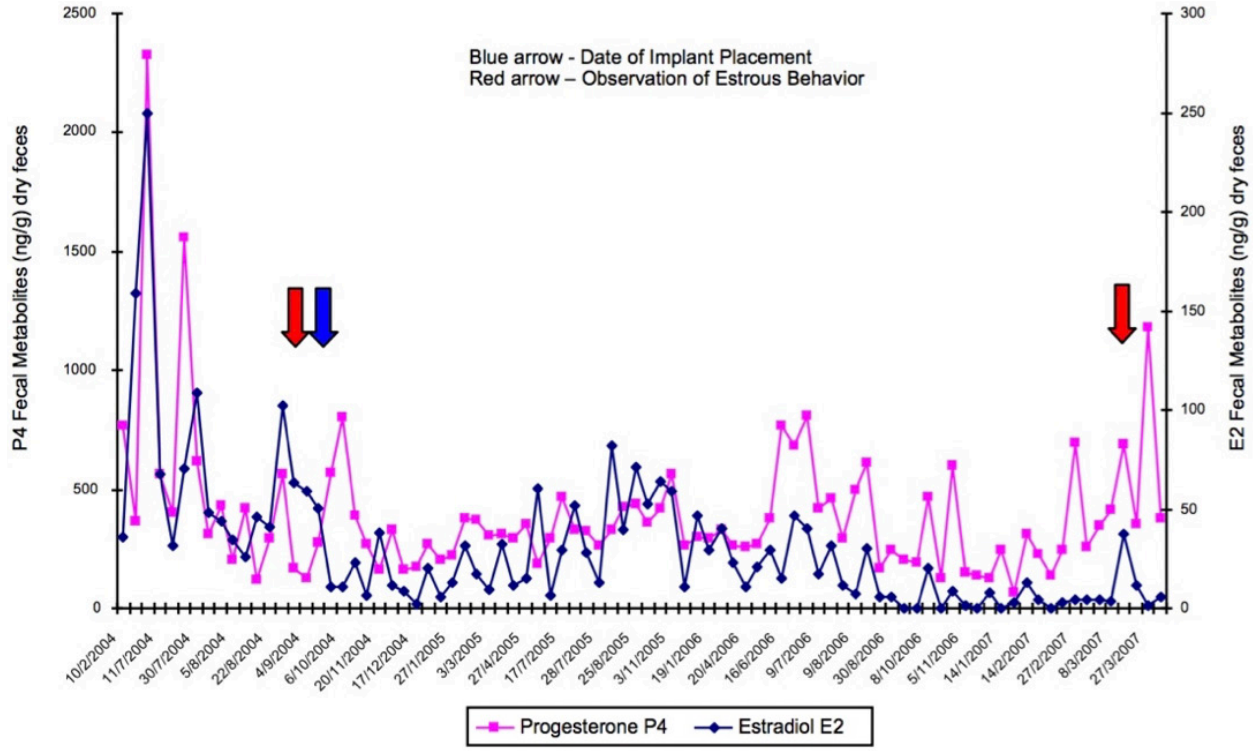

Figure 4. Fecal steroid metabolites profile of the lioness L4 (Panthera leo). Blue arrow—date of implant placement | red arrow—observation of estrous behavior.

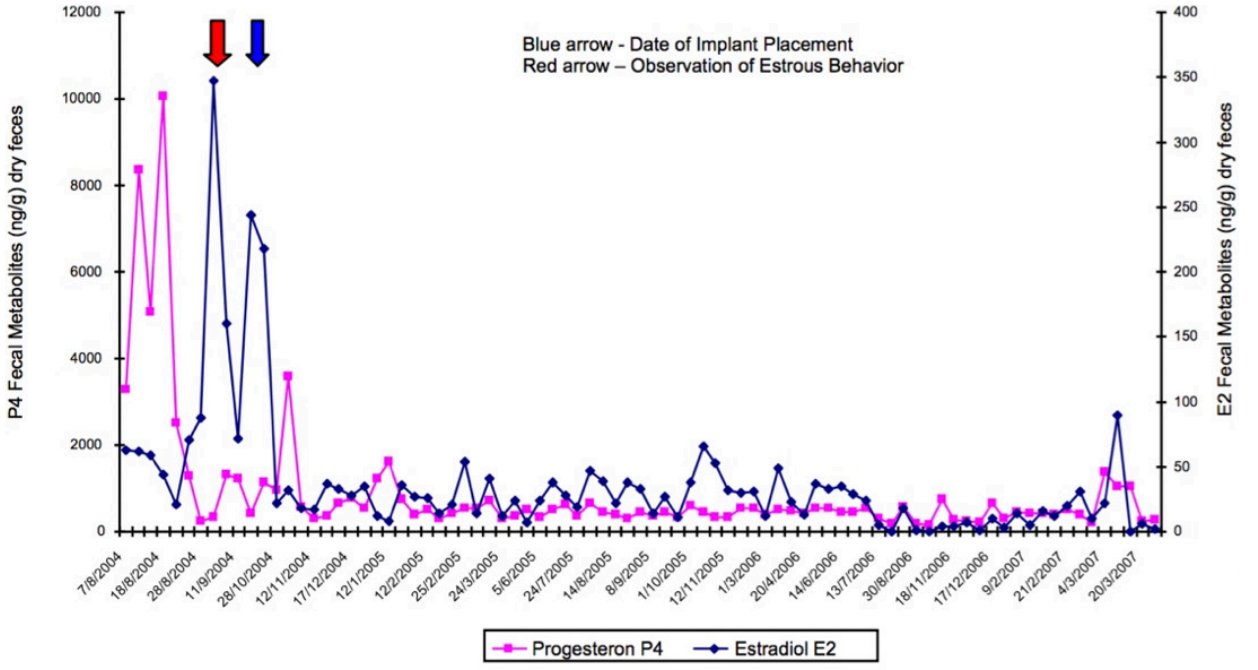

Figure 5. Fecal steroid metabolites profile of the lioness L5 (Panthera leo). Blue arrow—date of implant placement | red arrow—observation of estrous behavior.

DISCUSSION

In the face of the large loss of habitat due to human encroachment and fragmentation, some species become overabundant through human ineptitude. Indeed, humans often attempt to create conditions that favor the proliferation of one species over their competitors. Protected parks and reserves provide animals with an environment that is abundant in resources and predator-free, conditions that allow for unchecked reproduction. As a result, endangered species undergo a localized population explosion that can have detrimental effects on the flora and fauna of the reserve, putting other species at risk; thus, affecting the ecosystem in the same manner as do invasive species (Grandy & Rutberg 2002; Jewgenow et al. 2006).

Wildlife population control by means of contraception has become extremely important, especially for a number of wild carnivores. Population management and alternative noninvasive contraceptive methods have been studied extensively over the last two decades (Rosenfield 2016). Whereas ovariohysterectomy or ovariectomy alone has been the method of choice for most domestic cats (Munson 2006), for reproductive management of threatened or endangered species like the African Lion, a reversible method is desired. While lions can reach high densities inside reserves (Packer et al. 2013), they tend to fare poorly outside protected areas, where they are often the first large carnivore species to disappear (Woodroffe 2001).

The GnRH analog deslorelin, a long-acting biocompatible subcutaneous implant that suppresses specific pituitary functions, has been recommended as reversible contraception (D'Occhio et al. 2002). The increased release of GnRH into the portal vessels which connect the hypophysis to the pituitary gland results in an increased secretion of the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, regulate gonadal functions (Conn & Crowley 1994). With continuous exposure to high concentrations of GnRH, the number of cell surface receptors at the portion of the adenohypophysis – responsible for FSH/LH synthesis and release – gradually decreases (Melson et al. 1986) with a concomitant desensibilization effect of gonadotroph cells on GnRH (D'Occhio & Kinder 1995). By this type of mechanism, known as receptor down-regulation, chronic treatment with a GnRH agonist prevents the pulsatile release of FSH, as well as LH (Gong et al. 1995) and the pre-ovulatory surge of LH secretion (D'Occhio & Kinder 1995).

The absence of surge-releases of LH in females treated with a GnRH agonist have led to studies being conducted on the potential long-acting contraceptive effects of the GnRH agonist bioimplant by preventing follicular development and ovulation, and consequently, pregnancies (D'Occhio & Kinder 1995). In addition, the development of a noninvasive fecal steroid assay for assessing the ovarian function of felid species in combination with behavioral studies makes it possible to systematically study various aspects of reproduction (Brown et al. 1994, 2001; Graham et al. 2006). Therefore, the goal of the present study is to use the noninvasive fecal steroid assay associated with behavioral estrus to evaluate the efficiency of chronic treatments with the GnRH agonist bioimplants to suppress cyclic ovarian activity in African lionesses.

The inhibitory effects of ovarian activities, such as the arrest of ovulation caused by desensibilization to endogenous GnRH, provide opportunities to evaluate a GnRH agonist bioimplant as a potential antifertility agent in mammals. In the present experiment, seven lionesses were implanted with a 9.4 mg deslorelin to monitor ovarian function for 36 months. Fecal steroid assay and estrus behavioral observation were the monitoring methods used. Our findings suggest that the GnRH agonist deslorelin suppresses ovarian activity in African lionesses for prolonged periods of time. In fact, no behavioral estrus was noted until 22 months post-implantation. In the 22nd, 31st, and 33rd month, behavioral estrus was noted in three of the lionesses, while the fourth lioness exhibited increased estrogen concentrations and a consequent surge in progesterone level that corresponded to the resumption of ovarian activity, including ovulation, in addition to behavioral estrus.

One lioness died 15 months after the beginning of the experiment without demonstrating any estrus signs nor a rise in progesterone level. On the other hand, the estrogen concentration increased 12 months after the implantation, indicating that the ovarian activity may have re-started. Surprisingly, in a single female, neither estrus behavior nor a rise in fecal progesterone concentration was noted up to the end of the experiment.

Various behavioral activities that characterize estrus in lions appear to be common in several feline species, such as the domestic cat (Graham et al. 2000; Pelican et al. 2005), Jaguar (Wildt et al. 1979; Jorge-Neto et al. 2018a), Siberian Tiger (Seal et al. 1987), Snow Leopard (Schmidt et al. 1993), and Cheetah (Wielebnowski & Brown 1998), possibly serving as indicators of physiological estrus in these animals (Umapathy et al. 2007). It, however, remains unclear why behavioral estrus was observed in two of the lionesses without a rise in fecal estrogen and

progesterone metabolites concentration. Ovulation in *Panthera* genus species is triggered by copulation or sensorial stimulation (Jorge-Neto et al. 2020). Therefore, the lack of ovulation observed during this study may demonstrate a estrus detection failure or a compromised ovarian function. It could also be hypothesized that, in these cases, ovarian activity may have re-started and estradiol concentration increased, resulting in the stimulation of behavioral estrus, although, not enough to trigger the cascade of events to reach ovulation.

The fact that neither estrus behavior nor a rise in fecal progesterone concentration was noted in one of the lionesses up to the end of the experiment raises concern. For contraception to be successful for population control, especially in endangered animals, it must not only be safe, effective, and long-acting but also reversible (Castle & Dean 1996).

To date, deslorelin has been used in captive-held wild felines, such as cheetahs (Bertschinger et al. 2001), leopards (Bertschinger et al. 2002) and lions (Bertschinger et al. 2008), without showing any adverse effects. Conversely, in domestic cats, a 6mg implant has been shown to suppress ovarian follicular activity for between four and 14 months, however, until the end of the study period, eight out of ten cats did not fully return to normal ovarian cyclicity (Munson et al. 2001). Moreover, dosages of 12 or 15 mg deslorelin induced contraceptive effects for 12–18 months (Bertschinger et al. 2002). The implant used in this study (9.4mg) has a matrix without sodium acetate anhydrous, that allows slow liberation of the deslorelin, maintaining contraceptive effects for much longer periods, making it impossible to compare the effectiveness of this dosage in relation to the duration of previous products. It has been reported that the effectiveness duration of Suprelorin in wild felids is, on average, twice that prescribed by the manufacturer in dogs, which means that the 9.4mg implant with a minimum effectiveness of 12 months is generally effective for approximately 24 months (Asa et al. 2012). Our findings show a ceasing of ovarian activity of 28.67 ± 5.86 months, which corroborates those found by Bertschinger et al. (2008), in which implants were effectively in lionesses for a period of ~30 months or longer. The reversal time (or duration of efficacy) is variable between species and individuals, probably due to the singularity in the metabolism of deslorelin or the ability to recover from down-regulation (Asa et al. 2012). The findings suggest that long-term treatment with deslorelin may have variability regarding the duration of contraception among individuals due to several factors, including drug/matrix used; genetic and/or environmental influences. The disadvantage of using Suprelorin is the inability to safely predict the duration of effectiveness and the return of ovarian activity, being a problem when there is interest in using these females in conservation programs.

An extensive study using 140 implants (Suprelorin) on 14 species of wild felids, including 59 lionesses, was conducted by the North American Association of Zoos and Aquariums (AZA) and showed no side effects of deslorelin treatment (Asa et al. 2012). Bertschinger et al. (2008) used deslorelin treatment in 23 captive and 40 free-ranging lionesses (*P. leo*) and four captive tigers (*P. tigris*) in South Africa and did not observe any side effects in any females, including some treated four or five times for 5–8 years period. In domestic cat females the use of Suprelorin appears to be a convenient, efficient and safe contraception method, demonstrating female fertile matting after approximately two years post-treatment and no side effects (Fontaine 2015).

Prior to the occurrence of a GnRH agonist antifertility effect, there is an acute phase (D'Occhio et al. 2002; Rosenfield 2016) in which the secretion of LH and FSH increase sharply (Gong et al. 1995, 1996), leading to a corresponding estrus response (Wright et al. 2001). In the present study, shortly after placing the implant, two lionesses exhibited behavioral estrus, and an upsurge of ovarian activity was observed, as demonstrated by increases in the estrogen concentration. A rise in progesterone concentration was noted in two other females. As noted, the treatment-induced behavioral estrus signs without the accompanying rise in progesterone, observed in the first two females could be attributed to a copulation failure rather than compromised ovarian function. As reported in other works, after an initial GnRH treatment, lionesses and cheetahs may exhibit signs of estrus behavior and become attracted to males for a few days, although mating may not occur (Bertschinger et al. 2002).

Conversely, in animals in which a rise in progesterone concentration was noted but no behavioral estrus signs could be observed, a failure in observing estrus signs, a spontaneous ovulation – or sensorial stimuli štriggering ovulation – may have occurred. Spontaneous ovulation has been previously reported in some felines including all *Panthera* species, such as the Leopard (Schmidt et al. 1988), Snow Leopard (Brown et al. 1995), Tiger (Graham et al. 2006), Jaguar (Barnes et al. 2016; Gonzalez et al. 2017) and African Lion (Schramm et al. 1994) while sensorial stimulation has induced ovulation in Jaguars (Jorge-Neto et al. 2020). In one lioness, shortly after placing the implant, no behavioral estrus signs were observed, nor was there a rise in progesterone levels. This may be due

to the presence of active luteal tissue from a previous follicular cycle and/or due to individual variations.

Our results reinforce the importance of using non-invasive monitoring as an alternative for hormonal assessment, especially in wild animals. Blood collection is not only a stressful event and can itself cause changes in hormonal concentrations (Sheriff et al. 2011), but also does not allow successive collections for longer studies, such as monitoring of ovarian cyclicity (Sgai et al. 2015). Many studies in several species have been developed and validated for the longitudinal measurement of hormonal metabolites, both for glucocorticoids (Sinhorini et al. 2020) and steroids, enabling effective reproductive monitoring with fecal matrix (Monfort et al. 1997; Van Meter et al. 2008). These studies demonstrated efficient results without the need to perform a serum endocrine evaluation.

In conclusion, long-term treatment with a GnRH agonist has been shown to be extremely effective in inhibiting the synthesis and liberation of FSH and LH from the pituitary, and as a result, ceasing ovarian activity in female African lions for 28.67 ± 5.86 months. The duration of contraception, however, may vary among individuals, with the added risk of some females not returning to normal ovarian activity, rendering that female infertile. It is strongly suggested that further studies investigate the long-term antifertility effects of GnRH agonists in this species.

REFERENCES

Asa, C., S. Boutelle, & K. Bauman (2012). AZA Wildlife Contraception Center Programme for Wild Felids and Canids. *Reproduction in Domestic Animals* 47(SUPPL. 6): 377–380. https://doi.org/10.1111/rda.12004

Barnes, S.A., J. Andrew Teare, S. Staaden, L. Metrione & L.M. Penfold (2016). Characterization and manipulation of reproductive cycles in the jaguar (*Panthera onca*). *General and Comparative Endocrinology* 225: 95–103. https://doi.org/10.1016/j.ygcen.2015.09.012

Bertschinger, H.J., C.S. Asa, P.P. Calle, J.A. Long, K. Bauman, K. DeMatteo, W. Jöchle, T.E. Trigg & A. Human (2001). Control of reproduction and sex related behaviour in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. *Journal of Reproduction and Fertility (Supplement)* 57: 275–283.

Bertschinger, H.J., M.A. de B.V. Guimarães, T.E. Trigg & A. Human (2008). The use of deslorelin implants for the long-term contraception of lionesses and tigers. *Wildlife Research* 35(6): 525. https://doi.org/10.1071/WR07141

Bertschinger, H.J., T.E. Trigg, W. Jöchle & A. Human (2002). Induction of contraception in some African wild carnivores by downregulation of LH and FSH secretion using the GnRH analogue deslorelin. *Reproduction (Supplement)* 60: 41–52.

Brown, J.L., D.E. Wildt, L.H. Graham, A.P. Byers, L. Collins, S. Barrett & J. Howard (1995). Natural versus chorionic gonadotropin-induced ovarian responses in the Clouded Leopard (*Neofelis nebulosa*) assessed by fecal steroid analysis. *Biology of Reproduction* 53(1): 93–102. https://doi.org/10.1095/biolreprod53.1.93

Brown, J.L., L.H. Graham, N. Wielebnowski, W.F. Swanson, D.E. Wildt & J.G. Howard (2001). Understanding the basic reproductive biology of wild felids by monitoring of faecal steroids. *Journal of Reproduction and Fertility (Supplement)* 57: 71–82.

Brown, J.L, S.K. Wasser, D.E. Wildt & L.H. Graham (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biology of Reproduction* 51(4): 776–786. https://doi.org/10.1095/biolreprod51.4.776

Castle, P.E. & J. Dean (1996). Molecular genetics of the zona pellucida: implications for immunocontraceptive strategies. *Journal of Reproduction and Fertility (Supplement)* 50: 1–8.

Conn, P.M. & W.F. Crowley (1994). Gonadotropin-releasing hormone and its analogs. *Annual Review of Medicine* 45(1): 391–405. https://doi.org/10.1146/annurev.med.45.1.391

D'Occhio, M.J. & J.E. Kinder (1995). Failure of the LH-releasing hormone agonist, deslorelin, to prevent development of a persistent follicle in heifers synchronized with norgestomet. *Theriogenology* 44(6): 849–857. https://doi.org/10.1016/0093-691X(95)00270-I

D'Occhio, M.J., G. Fordyce, T.R. Whyte, T.F. Jubb, L.A. Fitzpatrick, N.J. Cooper, W.J. Aspden, M.J. Bolam & T.E. Trigg (2002). Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. *Animal Reproduction Science* 74(3–4): 151–162. https://doi.org/10.1016/S0378-4320(02)00189-6

Fontaine, C. (2015). Long-term contraception in a small implant. *Journal of Feline Medicine and Surgery* 17(9): 766–771. https://doi.org/10.1177/1098612X15594990

Gong, J.G., B.K. Campbell, T.A. Bramley, C.G. Gutierrez, A.R. Peters & R. Webb (1996). Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously Infused with a gonadotropin-releasing hormone agonist. *Biology of Reproduction* 55(1): 68–74. https://doi.org/10.1095/biolreprod55.1.68

Gong, J.G., T.A. Bramley, C.G. Gutierrez, A.R. Peters & R. Webb (1995). Effects of chronic treatment with a gonadotrophin-releasing hormone agonist on peripheral concentrations of FSH and LH, and ovarian function in heifers. *Reproduction* 105(2): 263–270. https://doi.org/10.1530/jrf.0.1050263

Gonzalez, S.J., J.G. Howard, J. Brown, H. Grajales, J. Pinzón, H. Grajales, J. Pinzón, H. Monsalve, M.A. Moreno & C.J. Escobar (2017). Reproductive analysis of male and female captive jaguars (*Panthera onca*) in a Colombian zoological park. *Theriogenology* 89: 192–200. https://doi.org/10.1016/j.theriogenology.2016.09.049

Graham, L.H., A.P. Byers, D.L. Armstrong, N.M. Loskutoff, W.F. Swanson, D.E. Wildt & J.L. Brown (2006). Natural and gonadotropin-induced ovarian activity in tigers (*Panthera tigris*) assessed by fecal steroid analyses. *General and Comparative Endocrinology* 147(3): 362–370. https://doi.org/10.1016/j.ygcen.2006.02.008

Graham, L.H., W.F. Swanson & J.L. Brown (2000). Chorionic gonadotropin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. *Theriogenology* 54(7): 1117–1131. https://doi.org/10.1016/S0093-691X(00)00420-9

Grandy, J.W. & A.T. Rutberg (2002). An animal welfare view of wildlife contraception. *Reproduction (Cambridge, England) Supplement* 60: 1–7.

Jewgenow, K., M. Dehnhard, T.B. Hildebrandt & F. Göritz (2006). Contraception for population control in exotic carnivores. *Theriogenology* 66(6–7): 1525–1529. https://doi.org/10.1016/j.theriogenology.2006.01.027

Jorge-Neto, P.N., T.C. Luczinski, G.R. Araújo, J.A. Salomão Júnior, A.S. Traldi, J.A.M. Santos, L.A. Requena, M.C.M. Gianni, T. Deco-Souza, C.S. Pizzutto & H. Baldassarre (2020). Can jaguar (*Panthera onca*) ovulate without copulation? *Theriogenology* 147: 57–61. https://doi.org/10.1016/j.theriogenology.2020.02.026

Jorge-Neto, P.N., C.S. Pizzutto, G.R. Araújo, T. Deco-Souza, L.C. Silva, J.A. Salomão Jr. & H. Baldassare (2018a). Copulatory behavior of the Jaguar Panthera onca (Mammalia: Carnivora: Felidae). *Journal of Threatened Taxa* 10(15): 12933–12939. https://doi.org/10.11609/jott.4218.10.15.12933-12939

Jorge Neto, P.N., L.A. Requena, C.S. Pizzuto & H. Baldassarre (2018b).

Laparoscopic Ovum Pick-Up (LOPU): From animal production to conservation. *SPERMOVA* 8(1): 61–67. https://doi.org/10.18548/aspe/0006.07

Melson, B.E., J.L. Brown, H.M. Schoenemann, G.K. Tarnavsky & J.J. Reeves (1986). Elevation of serum testosterone during chronic LHRH agonist treatment in the bull. *Journal of Animal Science* 62(1): 199–207. https://doi.org/10.2527/jas1986.621199x

Monfort, S.L., S.K. Wasser, K.L. Mashburn, M. Burke, B.A. Brewer & S.R. Creel (1997). Steroid metabolism and validation of noninvasive endocrine monitoring in the African Wild Dog (*Lycaon pictus*). *Zoo Biology* 16(6): 533–548. https://doi.org/10.1002/(SICI)1098-2361(1997)16:6<533::AID-ZOO6>3.0.CO;2-B

Munson, L. (2006). Contraception in felids. *Theriogenology* 66(1): 126–134. https://doi.org/10.1016/j.theriogenology.2006.03.016

Munson, L., J.E. Bauman, C.S. Asa, W. Jöchle & T.E. Trigg (2001). Efficacy of the GnRH analogue deslorelin for suppression of oestrous cycles in cats. *Journal of Reproduction and Fertility. Supplement* 57: 269–273.

Packer, C., A. Loveridge, S. Canney, T. Caro, S.T. Garnett, M Pfeifer, K.K. Zander, A. Swanson, D. MacNulty, G. Balme, H. Bauer, C.M. Begg, K.S. Begg, S.Bhalla, C. Bissett, T. Bodasing, H. Brink, A. Burger, A.C. Burton, B. Clegg, S. Dell, A. Delsink, T. Dickerson, S.M.Dloniak, D. Druce, L. Frank, P. Funston, N. Gichohi, R. Groom, C. Hanekom, B. Heath, L. Hunter, H.H. DeIongh, C.J. Joubert, S.M. Kasiki, B. Kissui, W. Knocker, B. Leathem, P.A. Lindsey, S.D. Maclennan, J.W. McNutt, S.M. Miller, S. Naylor, P. Nel, C. Ng'weno, K. Nicholls, J.O. Ogutu, E. Okot-Omoya, B.D. Patterson, A. Plumptre, J. Salerno, K. Skinner, R. Slotow, E.A. Sogbohossou, K.J. Stratford, C. Winterbach, H. Winterbach, S. Polasky (2013). Conserving large carnivores: dollars and fence. *Ecology Letters* 16(5): 635–641. https://doi.org/10.1111/ele.12091

Pelican, K.M., J.L. Brown, D.E. Wildt, M.A. Ottinger & J.G. Howard (2005). Short term suppression of follicular recruitment and spontaneous ovulation in the cat using levonorgestrel versus a GnRH antagonist. *General and Comparative Endocrinology* 144(2): 110–121. https://doi.org/10.1016/j.ygcen.2005.04.014

Rosenfield, D.A. (2016). Wildlife population control comprehensive and critical literature review on contraceptive methods in wildlife - mammals. Universidade de São Paulo, São Paulo, 219pp. https://doi.org/10.11606/D.10.2016.tde-05102016-134221

Schaller, G.B. (1972). Behavior within the group, pp. 83–166. In: Schaller, G.B. (ed.). *The Serengeti Lion. A study of predator-prey relations*. University of Chicago Press, Chicado, 504pp.

Schmidt, A.M., D.L. Hess, M.J. Schmidt & C.R. Lewis (1993). Serum concentrations of oestradiol and progesterone and frequency of sexual behaviour during the normal oestrous cycle in the snow leopard (*Panthera uncia*). *Reproduction* 98(1): 91–95. https://doi.org/10.1530/jrf.0.0980091

Schmidt, A.M., D.L. Hess, M.J. Schmidt, R.C. Smith & C.R. Lewis (1988). Serum concentrations of oestradiol and progesterone, and sexual behaviour during the normal oestrous cycle in the leopard (*Panthera pardus*). *Journal of Reproduction and Fertility* 82(1): 43–49. https://doi.org/10.1530/jrf.0.0820043

Schramm, R.D., M.B. Briggs & J.J. Reeves (1994). Spontaneous and induced ovulation in the lion (*Panthera leo*). *Zoo Biology* 13(4): 301–307. https://doi.org/10.1002/zoo.1430130403

Seal, U.S., R.L. Tilson, E.D. Plotka, N.J. Reindl & M.F. Seal (1987). Behavioral indicators and endocrine correlates of estrus and anestrus in Siberian tigers, pp. 244–250. In: Tilson, R. & U. Seal (Eds.). *Tigers of The World: The Biology, Biopolitics, Management, And Conservation of An Endangered Species*. Noyes Publications, Park Ridge, 540pp.

Sgai, M.G.F.G., C.S. Pizzutto, P. Viau, C.A. Oliveira & M.A. de B.V Guimarães (2015). Estudo endócrino reprodutivo e do comportamento sócio-sexual de sagui-de-tufo-preto (*Callithrix penicillata*) mantido em cativeiro. *Pesquisa Veterinária Brasileira* 35(3): 304–310. https://doi.org/10.1590/S0100-736X2015000300015

Sheriff, M.J., B. Dantzer, B. Delehanty, R. Palme & R. Boonstra (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166(4): 869–887. https://doi.org/10.1007/s00442-011-1943-y

Sinhorini, J.A., C.S. Pizzutto & R. Palme (2020). ACTH Stimulation Induced Self-Mutilation Behavior in the Golden Conure (*Guaruba guarouba*). *Animals* 10(3): 418. https://doi.org/10.3390/ani10030418

Umapathy, G., S.D. Sontakke, K. Srinivasu, T. Kiran, S.D. Kholkute & S. Shivaji (2007). Estrus behavior and fecal steroid profiles in the Asiatic lion (*Panthera leo persica*) during natural and gonadotropin-induced estrus. *Animal Reproduction Science* 101(3–4): 313–325. https://doi.org/10.1016/j.anireprosci.2006.09.014

Van Meter, P.E., J.A. French, K. Bidali, M.L. Weldele, J.L Brown & K.E. Holekamp (2008). Non-invasive measurement of fecal estrogens in the spotted hyena (*Crocuta crocuta*). *General and Comparative Endocrinology* 155(2): 464–471. https://doi.org/10.1016/j.ygcen.2007.08.002

Wielebnowski, N. & J.L. Brown (1998). Behavioral correlates of physiological estrus in cheetahs. *Zoo Biology* 17(3): 193–209. https://doi.org/10.1002/(SICI)1098-2361(1998)17:3<193::AID-ZOO4>3.0.CO;2-4

Wildt, D.E., C.C. Platz, P.K. Chakraborty & S.W.J. Seager (1979). Oestrous and ovarian activity in a female jaguar (*Panthera onca*). *Reproduction* 56(2): 555–558. https://doi.org/10.1530/jrf.0.0560555

Woodroffe, R (2001). Strategies for carnivore conservation: lessons from contemporary extinctions, pp. 61–92. In: Gittleman, J.L., S.M. Funk, D.W. MacDonald, & R.K. Wayne (eds.). *Carnivore conservation*. Cambridge University Press, 690pp.

Woodroffe, R. & L.G. Frank (2005). Lethal control of African Lions (*Panthera leo*): local and regional population impacts. *Animal Conservation* 8(1): 91–98. https://doi.org/10.1017/S1367943004001829

Wright, P.J., J.P. Verstegen, K. Onclin, W. Jöchle, A.F. Armour, G.B. Martin & T.E. Trigg (2001). Suppression of the oestrous responses of bitches to the GnRH analogue deslorelin by progestin. *Journal of Reproduction and Fertility. Supplement* 57: 263–268.

Resumo: Com o objetivo de avaliar a eficiência de um tratamento contraceptivo para a supressão cíclica ovariana em leoas africanas (*Panthera leo*) usando um bioimplante com agonista GnRH, foram utilizados ensaios não invasivos de esteroides fecais associados à observação de comportamento estral pelo período de 36 meses. Cinco fêmeas adultas em cativeiro, mantidas com um macho vasectomizado, receberam subcutaneamente um implante de 9,4mg de acetato de deslorelina. O tratamento inicialmente estimulou o comportamento estral, juntamente com a atividade ovariana, demonstrada pelo aumento de estrogênio em duas leoas. Um aumento na concentração de progesterona em outros dois animais sugeriu uma possível ovulação induzida pelo tratamento. Após o período inicial, a deslorelina impediu a atividade ovariana por pelo menos 22 meses. Duas fêmeas exibiram sinais de estro comportamental após 22 e 31 meses. Uma terceira leoa com aumento da concentração de estrogênio não apresentou sinais comportamentais de estro ou consequente aumento de progesterona até 33 meses após o implante, sugerindo uma possível retomada da atividade ovariana. Uma fêmea não exibiu nenhum sinal de estro comportamental nem um aumento nos níveis de esteroides após o estro "induzido pelo tratamento" durante todo o experimento (36 meses). Uma leoa morreu após 15 meses sem exibir sinais de estro ou um aumento no nível de progesterona. No entanto, a concentração de estrogênio aumentou 12 meses após o implante, sugerindo a retomada da atividade ovariana. O estudo mostrou que o tratamento a longo prazo com um agonista da GnRH pode ser extremamente eficaz como tratamento contraceptivo em leoas africanas; no entanto, a duração da contracepção pode variar entre os indivíduos e pode assumir o risco de perda permanente da atividade ovariana normal.

Author contributions: DPAF Braga, CS Pizzutto and MABV Guimarães conceived, designed, and directed the study. DPAF Braga, CS Pizzutto and PF Viau performed the experiments. SHR Correa, CA Oliveira, DPAF Braga, CS Pizzutto and MABV Guimarães analyzed and interpreted the data. DPAF Braga wrote the manuscript. CS Pizzutto, DA Rosenfield and PN Jorge-Neto critically revised the manuscript. All authors approved the manuscript for publication.

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