The past, present and future of hormonal contraceptive use in managed captive female tiger populations with a focus on the current use of deslorelin acetate

Contraceptive use in tigers

Amanda Guthrie, DVM, Dipl. A.C.Z.M., Dipl. E.C.Z.M. (ZHM), MRCVS, Taina Strike BVSc MSc (WAH) Dipl. E.C.Z.M. (ZHM) MRCVS, Stuart Patterson, BVetMed, MSc, MVetSci, PhD, MRCVS, Corinne Walker, BSc (Hons), Veronica Cowl, PhD, Ashley D. Franklin, PhD, David M. Powell, PhD

Authors affiliations:

AG,TS Zoological Society of London, Regent’s Park, London, NW1 4RY, UK

SP,CW Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College, University of London, Hawkshead Lane, Hatfield AL9 7TA, UK

VC The North of England Zoological Society (Chester Zoo), Chester Zoo, Caughall Road, Upton-by-Chester, CH2 1LH, UK

VC The European Association of Zoos and Aquaria (EAZA), Artis Zoo, 100 HD Amsterdam, the Netherlands.

VC,TS The EAZA Reproductive Management Group, Chester Zoo, Caughall Road, Upton-by-Chester, CH2 1LH, UK

AF,DP Association of Zoos & Aquariums (AZA) Reproductive Management Center, Saint Louis Zoo, One Government Drive, Saint Louis, MO 63110, USA

Corresponding author: Amanda Guthrie, amanda.guthrie@zsl.org

Abstract:

 Tigers (*Panthera tigris spp*.) are endangered in the wild; ensuring sustainable insurance populations requires careful planning within zoological collections. In captive situations, contraceptives are often used to control breeding and ensure genetically viable populations that contain manageable numbers of animals; reversible contraceptives are ideal because they offer flexibility for breeding management. Historically, synthetic progestins, such as melengestrol acetate implants, were used in female tigers, but these are associated with an increased risk of reproductive pathology and subsequent infertility. Recent management advice to ex-situ collections has been to transition to the use of GnRH agonists, such as deslorelin acetate implants, which do not appear to have a similar risk of reproductive pathology but are associated with highly variable reversal times in exotic felids. Using data from 917 contraceptive records in female tigers captured by the Association of Zoos and Aquariums Reproductive Management Center and the European Association of Zoos and Aquaria Reproductive Management Group’s joint Contraception Database and from supplementary surveys, this study reviews the changing use of contraceptives in captive female tigers. The aim was to describe the historical and current use of contraceptives and provide a comprehensive assessment on the use of deslorelin implants, including data on product protocols, efficacy, pathology and reversibility. This study determined that current dose, frequency, reversibility, and anatomical placement sites of deslorelin implants are highly variable, indicating that specific, readily available, unified, evidence-based recommendations on the use of deslorelin would be useful for future contraceptive use in managed tiger populations.

Key words: evidence based veterinary medicine, felid, melengestrol acetate, *Panthera tigri*s *spp.*, welfare

Research Highlights:

* Deslorelin acetate appears to be safe, effective and reversible in female tigers
* Deslorelin acetate is associated with little pathology
* Time between deslorelin acetate treatment and return to fertility is highly variable in female tigers

Graphical Abstract: See Figure 1

**INTRODUCTION**

The tiger (*Panthera tigris spp.*) is classified as Endangered according to the International Union for Conservation of Nature (IUCN). *In situ* populations are heavily fragmented, and population sizes are decreasing (Goodrich et al., 2015). To mitigate against species extinction, zoos are actively involved in sustaining genetically healthy insurance populations through captive breeding and management (Stubbington, Fitzpatrick, Cook, 2019; Cook, 2019; Traylor-Holzer, 2019a, b, c; Wildt et al., 2010). The Association of Zoos and Aquariums (AZA) and the European Association of Zoos and Aquaria (EAZA) manage high priority captive populations using Species Survival Plans (SSPs) and European Endangered species Programs (EEPs), respectively. Of the six extant tiger subspecies, the AZA manages three species as SSPs; the EAZA manages two species as EEPs. Amur and Sumatran tigers are also part of Global Species Management Plans, whereby various regional zoo associations collaborate on the management of the species. In July 2018, the EEP managed 120 Sumatran tigers at 54 institutions and 234 Amur tigers at 93 institutions (Stubbington, Fitzpatrick, Cook, 2019; Cook, 2019). In 2019, the AZA managed 121 Amur tigers at 51 institutions, 57 Malayan tigers at 27 institutions and 78 Sumatran tigers at 29 institutions (Traylor-Holzer, 2019a, b, c).

Tigers can be physically separated to prevent pregnancy, however, this might not be logistically feasible; additionally, it is common throughout Europe to keep tigers of various ages housed together for perceived welfare and social benefits. Even where achievable, long-term separation, or any situation that results in repeated exposure to gonadal hormones without an intervening pregnancy, is associated with pathology leading to fertility issues in many species, including tigers (Penfold, Powell, Traylor-Holzer, Asa, 2014); recent studies in tigers demonstrate they exhibit spontaneous ovulation which can result in prolonged exposure to progesterone in solitary or non–pregnant females (Cabot, et al., 2020). Therefore, to reversibly regulate reproduction and maintain genetic diversity, whilst also ensuring a stable but manageable population size, hormonal contraceptives are frequently used in breeding programs. Historically, knowledge and specific recommendations regarding the use of contraceptive products and methods in wild animals has been poorly disseminated. The “Contraception Database” (CD) was created so that institutions housing captive wild animals could contribute contraceptive records and centralize this knowledge (Asa, Boutelle, and Bauman, 2012).

As different contraceptive products are available in different regions, the database is maintained by two entities; European and West Asian institutions submit data to the EAZA Reproductive Management Group (RMG) (EGZAC, 2020). Institutions in the rest of the world submit their data to the AZA Reproductive Management Center (RMC) (Franklin, McDonald, and Powell, 2018). Generally, records are submitted voluntarily to the database, apart from institutions in North America who, because of regulations surrounding the distribution and use of contraceptive products under the Investigational New Animal Drug process (Powell and Asa, 2019), must report their experiences in order to receive certain contraceptive products from the RMC.

The first reports of contraceptive use in zoo felids came in the early 1970s with the use of synthetic progesterone products (Seal, et al., 1976; Asa, 2005a), primarily melengestrol acetate (MGA). These products have since been associated with uterine and mammary pathology and infertility in a variety of carnivores (Moresco and Agnew, 2013; Munson, 2006; Munson, Gardner, Mason, Chassy, Seal, 2002; Kazensky, Munson, Seal, 1998; Harrenstien, Munson, Seal, 1996); this has resulted in greater interest in using gonadotropin releasing hormone (GnRH) agonist implants, including deslorelin acetate (DA) beginning in the early 2000s (Asa, et al., 2012).

GnRH agonist contraceptives work by temporarily suppressing the hypothalamo-pituitary-gonadal axis, down regulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), inhibiting the production of sex steroids, and are effective in both sexes (Bertschinger and Sills, 2013). In females, GnRH agonists are generally effective beginning three weeks after treatment (Cowl, Walker, Rambaud, 2018), but prior to downregulation they temporarily stimulate the reproductive system which can result in fertile estrus and ovulation (Ackerman, et al., 2012; Cowl, et al., 2018; Moresco and Agnew, 2013; Bertschinger, et al., 2001; Fontaine, 2015). Stimulation may be followed by a non–pregnant luteal phase or sustained progesterone elevation which could promote uterine pathology (Asa et al., 2012). In females, the DA induced estrus can be suppressed by prophylactic treatment with oral megestrol acetate (MA), a short-acting progestagen (Asa, et al., 2014; Asa, et al., 2012; Moresco, Dadone, Arble, Klaphake, Agnew, 2014; Penfold, et al., 2014). Guidelines for zoo felids produced by the AZA RMC (Felidae: Carnivore, 2020) and the EAZA RMG (EGZAC, 2020, Strike, 2020) recommend a daily oral MA treatment beginning 7 days prior to DA implantation through 7 days post-implantation for the first DA treatment; subsequent consecutive DA implants do not need further MA supplementation.

The most used DA product is Suprelorin® (Suprelorin®, Virbac S.A.,1`ere avenue 2065 m LID-06516 Carros, France, EU), which is licensed for inducing temporary infertility in male dogs and male ferrets. On the basis of its efficacy in many species and the absence of an association with reproductive pathologies, the subcutaneous biocompatible implant appeared to be an ideal agent for controlling reproduction in large predators (Munson, 2006; Trigg et al., 2001; Wright et al., 2001; Junaidi et al., 2003; Cowl et al., 2018). Suprelorin® is available in 4.7 mg and 9.4 mg implants manufactured to be effective in male dogs for 6 and 12 months, respectively, regardless of the number of implants used (Moresco and Agnew, 2013). The duration of efficacy in wild female canids and felids has been approximately twice the manufacturer-stated minimum reported for domestic male dogs (Asa, et al., 2012; Bertschinger, de Barros Vaz Guimaraes, Trigg, Human, 2008) and Suprelorin® has been associated with variable durations of action and reversal rates in female felids (Bertschinger, et al., 2008).

This project aims to investigate and describe the historical and current use of hormonal contraceptives, with a focus on MGA and DA as they have been the most used, in globally managed populations of captive female tigers. This study will describe the transition to DA, identify how DA is being used and generate unified recommendations for the use of DA moving forward. Records were reviewed for reports of pathology associated with contraceptives; all records for captive female tigers contained in the CD were evaluated, including records of surgical sterilization.

**MATERIALS AND METHODS**

**The Contraception Database**

This study focused only on female tigers. The global CD was searched in October 2019 for all contraceptive records in female tigers (*Panthera tigris spp.*) since the inception of the database in 1989 (Asa, et al., 2012). Information pertaining to the individual (species, birth date, institution, sex, location, body weight, previous reproductive history) and to the contraceptive record (start and end dates, product, dose, implant location, start and end dates of mate access, non-contraceptive effects, implant recovery, reversibility) were collected. If data were missing, such as birth date and reproductive history, supplementary data were obtained using the Zoological Information Management System (Species360; www.Species360.org).

To identify whether there were differences in contraceptive use between the regions, institutions were classified as either EAZA institutions (institutions in Europe and Western Asia) or AZA institutions (institutions in the rest of the world). Tigers are described as Amur, Bengal, Indochinese, Generic, Malayan, or Sumatran.

**The EEP survey**

Recording contraceptive data with the EAZA-RMG is not mandatory; some EEP animals may not have been included in the records. Therefore, in addition to the contraceptive records from the CD, an online survey was produced (SurveyMonkey®; SI, Appendix 1) and sent electronically to 148 European institutions holding female Amur and Sumatran tigers managed by the EEP. The survey asked about the reproductive and contraceptive history of intact, sexually mature (> 3 years old) female tigers, held in collections between January 1, 2007 through July 1, 2018. Tigers that did not fulfill these criteria were removed from the analyses. Only complete responses to the survey were considered; duplicate results from the survey and the CD were counted as a single record. Forty-four institutions responded, results from 38 institutions fit the inclusion criteria and were included in the analysis, resulting in a 25.7% institutional inclusion rate. These 38 institutions reported on 78 individual female tigers, only 21 of which (12 Amur and 9 Sumatran) were treated with hormonal contraception. The survey identified 20 unique individual tigers (38 contraceptive treatment bouts) that were not originally included in the CD and have been added to the overall dataset for EAZA tigers.

**Historical overview**

In total, 917 records from 482 female tigers were used to describe the historic trends in contraceptive use in captive tigers. When separated by region, the data reflect information regarding 125 female tigers housed within institutions in Europe between dates August 1986 through May 2019 (EAZA) and 337 female tigers from the rest of the world (AZA) between the dates of October 1975 through June 2019. Products were grouped by their main active ingredient, rather than trade name, as these may vary across regions. Surgical methods of sterilization were also included in this overview. The number of records using each active ingredient/method as well as the earliest and latest start dates for each category were evaluated.

**Evaluating DA implant use in captive tigers**

The use of GnRH agonists other than DA has not been described for tigers in the CD. In total, there were 170 DA records from 89 tigers (167 records from 86 female tigers). Treatment protocols were evaluated by examining implant location, dose used, number of repeat treatments, and whether separation of sexes or supplementation with oral MA had been used for the first treatment with DA. The mean treatment interval was calculated for tigers that received multiple, concurrent DA implants; only bouts with definitive beginning and end dates were included. Where subsequent bouts occurred, the end date of a previous bout was considered the same day as the beginning date of the immediately subsequent bout unless otherwise specified. Product efficacy, defined as the prevention of estrus and/or pregnancy, was assessed by examining the number of records in which no treatment failures were reported, including ongoing records. Failures were identified according to Cowl et al. (2018) whereby “true” failures are failures in which the product was ineffective at suppressing estrus, or conception, and institutional failures are failures that account from the incorrect use of the contraceptive product, for example by failing to use MA in females at the beginning of the contraceptive bout. Total rates of efficacy were calculated, excluding records in which institutional failures had occurred.

The reversibility of DA implants was evaluated; this includes “full” reversals in which the contracepted individual produces offspring, including live and stillbirths, as well as partial reversals, which include behavioral (resumption of estrus and reproductive behaviors), or physiological reversals (sex steroids are no longer suppressed), but not resulting in detected pregnancies. Reversal time is calculated as the time from implant insertion to the time of conception of offspring (full reversal) or to the time of the resumption of behavioral or physiological indicators of fertility (partial reversal). For full reversals, only individuals that were given the opportunity to breed were included, including females who were given a breeding opportunity but were removed from the breeding pool before reversing (through death or by receiving permanent non-breeding recommendations). Conception date was estimated based on a mean gestation length of 107 days (Sadleir, 1966, Graham, Goodrowe, Raeside, Liptrap, 1995; Graham, et al., 2006; Gu, Guo, Stott, Jiang, Jianzhang, 2016).

**Pathological and non-contraceptive issues associated with contraception use**

Cases of pathology and weight gain reported in the CD were reviewed for MGA and DA, comparing the number of individuals and types of pathology reported for each contraceptive treatment.

**Statistical analyses**

Descriptive data were primarily used to describe trends because of the amount of available data. To identify whether the number of DA implants used was associated with individual weight or tiger subspecies, linear mixed effect models (LMMs) were used in which the number of implants was the dependent variable, and either individual weight or subspecies the independent variable. Individual ID was included as a random factor. The Fisher’s exact test was used to examine the effect of implant removal, previous offspring, previous contraception, and the age of contraception on full reversals. Given the limited sample size, logistic regression to identify whether the age of contraception associated with full reversals was not possible. As such, age was treated as a categorical value and was also analyzed using the Fisher’s exact test.

LMMs were used to evaluate the influence of variables (age at contraception, implant removal, previous contraception, previous offspring, product type, and number of implants) on time to reversal. Data for full and partial reversals were analyzed separately. Age of contraception was treated as a continuous variable. Again, individual ID was the random factor. Significance of independent variables was assessed using ANOVA; Tukey post-hoc multiple mean comparison tests were carried out on significant variables. All analyses were carried out using R (R Development Core Team, 2013); LMMs were carried out using the lmer function in the *lme4* package (Bates, Machler, Bolker, Walker, 2015).

**RESULTS**

**Historical overview**

The most reported type of contraception in female tigers were synthetic progestins, constituting 77.1% of records (707 records). Of the progestins, MGA was most used (66% of records). The earliest reported contraceptives in female tigers were the progestin medroxyprogesterone acetate (MPA) in 1975 in AZA tigers and in 1984 in EAZA tigers. The most recent records of progestin use as a contraceptive in female tigers was MA in December 2017 in AZA, and the use of MPA in November 2016 in EAZA. The currently recommended contraceptive, DA, is reported as the contraceptive method in 18.2% of records (167 records: 70 AZA records and 97 EAZA records). Reported use of DA as a female contraceptive began in 2004 in EAZA and in 2005 in AZA institutions, with the most recent records occurring in 2019 in both EAZA and AZA, respectively (Figure 1).

Of 27 records describing MA use, only 6 AZA records (22%) and 1 EAZA record (3.4%) describe the use of MA as a sole contraceptive product, with treatments beginning in 1979 in AZA, and in 2003 in EAZA. The use of contraceptive products containing MA, in accordance with guidelines, as a supplement to suppress the initial stimulation phase caused by the DA implant is reported in 20 of 27 MA records (74.1%). The practice began in AZA in 2005, with EAZA institutions beginning to supplement DA implants in 2007. The last reported use of supplemental MA was reported in 2018 in AZA and in 2019 in EAZA; there are more recent initial bouts of DA that do not report the use of supplemental MA.

Surgical contraception was reported in 4.0% of records (37 records); 28 females were ovariohysterectomized (75.7%), six were hysterectomized (16.2%), and three received tubal ligations (8.1%). Reports of surgical sterilization began in 1980 with a female generic tiger who was hysterectomized in an AZA institution, and continued until 2016 with a female Amur tiger who was ovariohysterectomized in an EAZA institution. Medical issues including pyometra, a mineralized uterus, hip dysplasia, and uterine infections were cited as the cause for surgical sterilization in 32.4% of the surgical contraception records.

**The Present: current contraceptive use**

**Evaluating DA implant use in captive tigers**

In 99.4% of cases (166 of 167 treatment bouts), DA was effective at contracepting females, regardless of dose and product protocol. One possible failure was reported in an AZA female Sumatran tiger, who had been treated with 2 x 9.4mg implants in October 2006; no suppression was observed, as determined by fecal estradiol monitoring (personal communication).

The most reported implant placement site was between the scapulae (38.8%; Table 1). Several records had unknown implant placement sites in both datasets (28.2%). Excluding unknown locations, implant placement sites were more diverse in EAZA institutions than in AZA institutions, with 10 and 5 different placement sites reported in each dataset, respectively. Implant removal was uncommon, regardless of implant location or zoological region, and only occurred in 4.2% of records. Difficulty removing implants was only reported in two cases, in which only half of the placed implants (1 of 2) located at the base of the ear were located and removed.

When using DA, on average, each female received 1.91 ± 1.50 treatments **(**mean ± standard error, n=86, median =1). Overall, 53.5% (46 records) received only one treatment, 25.6% (22 records) received two treatments, 9.3% (8 records) received three treatments, 7.0% (6 records) received four treatments, 2.3% (2 records) received six treatments, one animal received eight treatments (1.2%) and one animal received nine treatments (1.2%). No animals received five or seven treatments; repeat treatments do not imply that the same dose was used for each bout.

Most tigers in AZA institutions received 3 x 9.4 mg implants (27.1%), while 42.3% of tigers in EAZA institutions received 2 x 9.4 mg implants (Table 2). The number of implants per treatment was not associated with subspecies (ANOVA: F(1,45) = 0.19, p = 0.70) or body weight (F(1,80) = 2.04, p = 0.08). In both regions, 9.4 mg implants (71.3%) were used more frequently than 4.7 mg implants (25.7%). In one AZA Sumatran tiger, a combination of 1 x 4.7 mg and 1 x 9.4 mg implants were administered.  The mean interval between repeat treatments with 4.7 mg implants was 10.7 months (range: 5.0-35.4, mode: 6.2, 12 tigers, 18 bouts) and 14.2 months with 9.4 mg implants (range: 5.5-31.4, mode: 11.5, 27 tigers, 45 bouts).

**Separation of the sexes or supplementation of the first bout of DA**

Of the 167 DA records, 85 records described a female’s first treatment with DA, or re-contraception with DA after the assumed expiration of previous implants. Separation of the sexes upon the placement of a female’s first DA implant occurred in 20 instances (23.5% of first DA records; 7 AZA; 13 EAZA). This practice occurs over a larger timespan in EAZA institutions; from 2004 until 2019 compared to 2007 to 2010 in AZA institutions.

Supplementation of the first DA bout with MA occurred in 16 records (18.8%; 10 AZA; 6 EAZA). This practice began in AZA institutions in 2005, with the most recent report of DA supplementation with MA in 2015, while in EAZA institutions; this occurred between 2007 and 2019. The most recent use of MA as a supplement does not coincide with the most recent first DA records in either region. In 3 records (3.5%; 1 AZA; 2 EAZA), MA products were used at the time of first contraception with DA, but guidelines were not followed for proper dosing and administration. In two records, MA had been used for two to five months prior to treatment with DA, and in one record, supplementary MA treatment ended on the day that DA was implanted.

In summary, correct protocols to suppress the stimulation phase or mitigate potential pregnancies as per AZA RMC and EAZA RMG felid guidelines were reportedly only followed in 42.4% of cases (36 records). Reproductive abnormalities were identified in three females with unsupplemented DA treatment: one record reported suspected reproductive pathologies in a female and one reported a presumed metritis. One report describes firm yellow masses on the ovaries three years after implant placement; records are insufficient to differentiate pathology from normal corpora lutea secondary to spontaneous ovulation in this case.

**The evaluation of present success: the reversibility of DA**

Rates of full reversal within the global dataset demonstrate that 55.6% of females that were given a breeding opportunity produced offspring following contraception (10 of 18 records: Table 3).  Mean time to conception for 4.7 mg implants was 50.7 months (range: 26.5 - 64.8 months; median: 55.7 months) and 51.9 months for 9.4 mg implants (range: 35.4 - 92.1 months; median: 45.1 months). However, for the female who conceived after 26.5 months, mate access was only given 13.5 months before the estimated date of conception. Within 4 years of implant placement, 20% of tigers implanted with 4.7 mg implants produced offspring, while 30.8% of females implanted with 9.4 mg implants reversed. In contrast, females who had been given the opportunity to breed but have not successfully fully reversed yet have been in a breeding situation for 112.9 – 145.2 months and females who had been given a breeding opportunity but died before conceiving offspring had been in a breeding situation for 17.7 – 149.0 months. Time to full reversal was not associated with the number of implants (ANOVA: F (1,7) = 1.48, p = 0.26) or product type (ANOVA: F (1,7) = 0.01, p = 0.94). Reversals were not influenced by previous contraception (Fisher’s exact test: p = 1.00), reproductive success (p = 0.63), the age of contraception (p = 0.74), nor by implant removal (p = 0.80).

Behavioral signs of estrus and male interest were observed as soon as 4.8 months after the insertion of 4 x 4.7mg implants in one case, which may suggest that the implants expired earlier than the manufacturer’s minimum duration of efficacy (6 months, Table 4). Potential early expiration was also observed in two other cases in which two females exhibited signs of behavioral estrus 5-7 months after the insertion of 3 x 4.7mg implants. The time taken to observe behavioral or physiological reversal was not associated with previous contraception (ANOVA: F(1,22) = 0.07, p = 0.79), previous reproductive success (F(1,23) = 0.44, p = 0.51), the age of contraception (F(1,23) = 0.79, p = 0.38), implant removal (F(1,8) = 1.25, p = 0.30), or the number of implants (F(1,22) = 3.91, p = 0.06). The time to partial reversal was however, significantly associated with product type (4.7 mg, 9.4 mg, or a combination of 4.7 and 9.4 mg implants; F(3,21) = 3.43, p = 0.04); time to partial reversal was only significantly higher in females implanted with 9.4 mg implants than in females implanted with 4.7 mg implants (9.4 mg – 4.7 mg: difference ± standard error: 16.59 ± 5.7 months, p = 0.03). Behavioral signs of estrus do not necessarily reflect ovulation nor reversal as deslorelin may be associated with infertile follicular growth associated with LH pulses (Cecchetto, et al., 2017). Behavioral signs of estrus should not be confused with implant failure in these instances.

**MGA associated pathology**

Within the dataset, 106 females were treated with MGA implants. Neoplasia was reported in 37 (34.9%) tigers of different subspecies, having experienced one to six bouts of treatment with MGA implants, for up to ten years of contraception. However, when implants are left in place, they may be active for a period longer than the expected expiration date; 76.8% of MGA records described the implants being removed. These tigers ranged in age from 5 to 20 years.

The greatest number of neoplasia diagnoses (20 animals; 74.0% of neoplasias) were reported in the mammary glands where mammary adenocarcinoma, with or without metastasis, was reported in 18 cases (48.6% of neoplasias); the remaining two cases were fibrocystic mammary dysplasia in one and unspecified mammary gland tumors in another. The following reproductive tract pathologies were described in five tigers (4.7% of MGA contracepted tigers): endometrial cancer; endometrial carcinoma; uterine adenocarcinoma with metastasis to kidney, bladder, pancreas, lung, and lymph nodes; and two cases of ovarian cancer. The mean age of tigers with reproductive and mammary neoplasia was 12.9 years. Other non-reproductive or unspecified neoplasia was reported in 13 tigers (12.3% of MGA contracepted tigers) with a mean age of 12.7 years.

Pyometra or endometritis was reported in 11 tigers of different subspecies (11.0% of MGA treated tigers), ranging from three to 18 years and after MGA treatments ranging from one to five bouts; the average age was 10.5 years. The youngest animal was a three-year-old tiger that developed endometritis six months after her first MGA implant and the oldest animal was 18 years old and had been treated with three MGA implants over six years. Four animals showed a pattern of endometritis or pyometra development after a first implant, regardless of their age. One animal had received five MGA implants over a five-year period prior to developing a uterine infection. Ovariohysterectomy was used to treat 45.0% (5/11) of these pyometra/endometritis cases, but it is unclear from the data set whether the remaining animals returned to reproductive health; four cases had concurrent mammary adenocarcinomas.

Mineralized uterine tissue was reported in four animals (33.7%) and ovarian cysts in two animals (1.9%). One animal had spot bleeding throughout MGA contraception but there was no reproductive tract pathology reported when she was ovariohysterectomized.

Eight tigers of different species developed abscessation or infection at the site of the implant (7.5%). There did not appear to be any correlation between how many MGA implants had previously been inserted and the formation of an abscess. Abscesses appeared more common in younger animals with the average age being 6.4 years. Additionally, there were 48 instances of implant loss (45.3%), some recurring in the same animal; these were counted separately from the abscess cases.

**DA associated pathology**

Ninety-seven individuals treated with DA implants were included in the analysis of DA associated pathology. No reproductive tract or mammary neoplasms were reported in animals with a history of only DA contraception. However, an 11-year-old Malayan tiger that had three bouts of MGA implants over six years, followed by DA (preceded by MA treatment) was found to have an unspecified carcinoma on postmortem examination. An 11-year-old Indochinese tiger that had one bout of DA had an oral fibrosarcoma reported on postmortem examination.

A non-purulent vaginal discharge was described in a five-year-old Malayan tiger, five months after her first DA implant; the implant had not been preceded with MA treatment. The animal went on to have five subsequent implants with no further vaginal discharge.  Metritis was described in a 13-year-old Amur tiger after a single bout of DA, which was not preceded by MA treatment.

Unspecified reproductive pathologies were listed in a five-year-old Amur tiger after a single unsuppressed bout of DA; however, it was not clear whether these were found at postmortem or were in the form of a pyometra or endometritis. There were no reports of abscessation at the implant site and no reports of implant losses in animals that received DA contraception. Firm yellow masses were reported incidentally at postmortem on the ovary of a 20-year old Sumatran tiger that had been contracepted with DA three years prior.

**Weight gain**

 Body weight changes associated with MGA was reported in 23 records; 11 reported no weight gain (47.8%) and 12 reported weight gain (52.2%). There were 27 records that reported information about DA-associated weight change; 14 reported no weight gain (51.9 %) and 13 reported weight gain (48.1%).

**DISCUSSION**

The aim of this study was to examine whether perceived changes in the use of hormonal contraceptives in captive female tigers had truly occurred, to examine the manner and success with which these products are being used and to make recommendations for future guidance. This work demonstrates that since the early 2000s there has been an increasing use of the GnRH agonists at the expense of the synthetic progestins (Figure 1).  This study highlights a surprising range of protocols for the use of these drugs, despite the available guidelines from the AZA RMC and EAZA RMG. Although there is evidence of very few neoplasias associated with GnRH agonists, data on reversal effectiveness is still based upon small numbers. Here, these findings are discussed in relation to the management of captive tigers and considering the implications for future guidance in line with the principles of evidence-based veterinary medicine.

**The Past: the historic use of contraceptive products**

In the late 1990s and early 2000s, evidence began to emerge about infertility and uterine and mammary gland pathology associated with MGA contraception in female wild felids (Kazensky, et al., 1998; Munson, et al., 2002; McAloose, Munson, Naydan, 2007; Asa, et al., 2012). MGA does not suppress folliculogenesis in treated females and this continuous exposure to hormones results in cystic endometrial hyperplasia and subsequent infertility (Kazensky and Seal, 1998). These findings coincided with a lack of availability of MGA in Europe and resulted in a shift to consider other contraceptive products.

The shift to DA use occurred earlier in EAZA institutions than in AZA institutions, particularly as hormonal growth promotors were banned from use in production animals in the EU from 1988 (EEC Directive 1988/146/EC) and hormonal implants for veterinary treatments from 1996 (EEC Directive 96/22/EC). MGA became commercially unavailable in Europe in 1996 and DA in the form of Suprelorin® became commercially available in 2007 (European Medicines Agency, 2020). In the United States, the Suprelorin® implant is not FDA approved, but can be used under the Investigational New Animal Drug process (Powell and Asa, 2019).

**The Present: the current use of DA**

Current dosing recommendations are two or three implants for large felid species including tigers, preceded - and followed by - seven days of 2–5mg/kg oral MA and should be placed in a location, such as the inner leg or ventral to the ear, that ensures ease of removal. (Strike, 2020, Felidae: Carnivore, 2020, Cowl et al., 2018, Asa, et al., 2012, Lewis, 2016).

Although DA doses given to tigers were highly variable in this study, most records in the CD used 2-3 implants successfully in their tigers with low rates of failure, indicating that current guidelines are fit for purpose. The results of this study highlight that single implants have been effective in Amur, Generic, Indochinese, Malayan, and Sumatran tigers. In practice, single implants could be administered, but should only be done in conjunction with routine endocrine monitoring to ensure that the dose is effective. In humans, body weight and body mass index affect contraceptive efficacy (Kapp et al. 2015; Holt et al. 2002), therefore, the number of DA implants administered to tigers should also reflect individual body weight. Generally, two implants should be used in average sized tigers: three implants in very large or over conditioned animals.

Most implants were placed in the manufacturer’s recommended site, between the scapulae (Cowl, et al., 2018; Fontaine, 2015), and notably, no implants were removed in animals given a breeding opportunity. Extended periods of reproductive suppression with DA use and long times to reconception have previously been described in domestic and exotic felids (Bertschinger et al., 2008; Goericke-Pesch, 2017; Moresco, et al., 2014; Fontaine, 2015; Putman, et al., 2015; Cowl et al., 2018). This study did not find an association between implant removal and time to partial reversal and low rates of implant removal were identified. The lack of association between variables such as implant removal and full or partial reversals in this study may also, in part, be because of the small sample size available for analysis, highlighting the need for more complete and comprehensive data collection across zoological institutions. Previous studies have demonstrated that physical removal of both MGA and DA implants are associated with a faster return to reproductive cyclicity and fertility in exotic felids than leaving the implant in place to expire (Chuei, Asa, Hall-Woods, Ballou, Traylor-Holzer, 2007; Moresco et al., 2014). Both the AZA RMC and EAZA RMG recommend that contraceptive implants are removed to quicken the time to reversal if reversal is desired. DA implants were not designed to be removed and become porous and prone to breakage with time; it is recommended that implants are inserted carefully in locations with thin skin to prevent damage, facilitate removal and ensure efficacy (Cowl, et al., 2018, Fontaine, 2015, Moresco et al., 2014).

Contraception with DA was reversible, although this study, as well as others (Bertschinger, et al., 2008), identified variable rates to reversal, with the duration of effect lasting beyond the manufacturer’s minimum duration of efficacy of 6 or 12 months for 4.7 and 9.4 mg implants, respectively. While the mean time to conception following a final treatment bout with DA was over 3 years with 4.7 mg implants and 4 years with 9.4 mg implants, mean treatment intervals for 4.7, and 9.4 mg implants were 10.7 and 14.2 months, respectively. Thus, the results from this study indicate that institutions could delay retreatment for a maximum of 2 years following treatment with 4.7 mg implants, and for a maximum of 3 years with 9.4 mg implants. These intervals may still lead to unplanned pregnancies in some individuals as these intervals are based on average responses; in cases where institutions are concerned about unplanned pregnancies, they could, at minimum, continue replacing 4.7 mg implants every 12 months, and 2 years for 9.4 mg implants; this will reduce, but not eliminate, the risk of unplanned pregnancies. The use of DA in pre– or peri– pubertal mixed sex littermates may extend the period of time that animals can remain together and avoid unplanned pregnancies.

**Clinical and pathological considerations of DA**

While neoplasias were described in almost one third of female tigers treated with MGA implants, contrastingly, in DA treated tigers, there are no reports of pathology or adverse side effects associated with DA use alone (Bertschinger, et al., 2008), and there were no cases or trends in the CD that were concerning. However, DA use is relatively recent, and many treated animals are young and still alive; it may be too early to determine whether DA use is associated with long-term reproductive pathology, infertility, or other medical concerns in female tigers. Moreover, the health risks of not supplementing a female’s first bout of DA with oral MA need to be carefully evaluated. Treatment with DA alone was associated with an increased risk of endometrial hyperplasia in some carnivore species (Asa, et al., 2014); the risk may be similar in felids; treatment with MA reduces exposure to hormones during the initial stimulation phase. While it is recommended by the AZA RMC and EAZA RMG to supplement a female’s first bout of DA with oral MA to suppress the initial stimulation phase (Asa, et al., 2012; Strike, 2020; Moresco et al., 2014), this study determined that fewer than 20% of females were treated with MA prior to their first treatment with DA. Moreover, 23.4% individuals were separated from their mate after DA implantation but did not receive MA supplementation, thus preventing pregnancy, but not suppressing estrus, potential ovulation, and the possible negative side effects of the stimulation phase (such as a non-pregnant luteal phase or pyometra). There have been periods in Europe where veterinary formulations of MA were commercially unavailable and human preparations were difficult or impossible to obtain, which may explain some of these instances. While the incidence of pathologies was low in DA treated females, correct protocols should be applied to minimize potential health and welfare risks to the individual.

Weight gain was reported in nearly half the tigers treated with DA or MGA, although at slightly lower frequencies in DA treated tigers. Weight gain and increases in body fat are commonly associated with gonadectomy (equivalent to DA use) in domestic cats, although this can be controlled, to some degree, by managing diet (Larsen, 2017; Nguyen, et al., 2004). Weight gain is a commonly listed side effect of contraception use in exotic species (Gray and Cameron, 2010; GnRH agonist-Deslorelin, 2020), including MGA and DA (Bertschinger et al., 2008) and it has been found that the more successive DA implants a lioness (*Panthera leo*) had, the more likely weight gain was to be reported (McEvoy, et al., 2019). Information on the weight of contracepted animals was commonly missing from the database so weight gain may be an underreported side effect of contraception with DA. Weight gain and obesity may lead to other health concerns such as diabetes mellitus or worsening of degenerative conditions like osteoarthritis; the body weight of captive wildlife should be monitored and managed closely to optimize health and welfare.

**Other contraceptive methods**

As reversal rates are less variable in felids following contraception with progestin-based contraceptives than with DA (Chuei, et al., 2007; Asa, et al., 2012; Bertschinger, et al., 2008), short-term progestin use for short-term contraception could be considered in some cases (Felidae: Carnivore, 2020). Because of the risks to reproductive health, treatment should however, not exceed a period of two years, and should be followed by a pregnancy before resuming further progestin therapy (Munson, et al., 2002; Dematteo, 2005).

Surgical contraception has been used in animals with pathology, medical conditions, and in animals that will never be recommended to breed. In tigers, any surgical method that allows fertile cycles without intervening pregnancies should be avoided (Asa, 2005a; Penfold, et al., 2014), such as tubal ligation, which does not alter normal hormonal activity in the female, or vasectomy, which will not prevent copulation, but will prevent copulation from being fertile (Asa, Porton, and Calle, 2005c; Dematteo, 2005). If the permanent sterilization of an individual is desired, managers should use castration in males (which stops copulation and thus induction of ovulation in females), or ovariectomies or ovariohysterectomies in females (Strike, 2020) (Cabot et al 2020 ) also recommend this? .

**The Future: recommendations for hormonal contraceptive use**

Based on the results of this study and current knowledge, the following are recommendations for contraceptive use in captive tigers: treat with two (4.7 mg or 9.4 mg) DA implants (three for over conditioned animals); this should be combined with oral MA to suppress estrus after the stimulation period (Felidae: Carnivore, 2020; Lewis, 2016; Strike, 2020). Implants should be replaced every 12 months for 4.7 mg implants and every two years for 9.4 mg implants; utilizing these longer intervals between repeat treatment bouts may improve tiger welfare by reducing the number of anesthetic events required to maintain effective contraception. Implants should not be placed in the manufacturer’s recommended site, between the scapulae (Cowl, et al., 2018; Fontaine, 2015), as DA implants were not designed to be removed; but anatomical placement such as subcutaneously ventral to the ear, the inner area of the leg (medial, distal tibia), and the umbilical area may facilitate easy removal (Table 1) (Cowl, et al., 2018; Fontaine, 2015; Moresco et al., 2014). The removal of implants is recommended and will hasten time to fertility after contraception. Placing a microchip (transponder) at the site of the deslorelin implant may facilitate removal. Oral MA should be used at a dose of 2 mg/kg once daily, for 7 days prior to and after, only the first bout of DA, in all instances, to suppress the stimulation phase and prevent associated pathology. Synthetic progestins should be used when short-term reversible contraception is desired. When the reversal of DA contraception is desired, reversal times for 9.4 mg implants are a minimum of two years, likely longer, as has been demonstrated in African lions (Tables 3 & 4) (Bertschinger, et al., 2008; Putman, et al., 2015). Implants should be placed under general anesthesia, in order to ensure that the implant is not broken during placement; implants must be removed equally carefully, without crushing. In tigers, the data suggest that efficacy is longer than indicated by the manufacturer.

**Reproductive management and individual fertility**

Long-term population sustainability is a key component of captive breeding, yet, in the AZA an estimated 64% of populations will decline unless management changes are implemented including increased reproductive output (Che-Castaldo et al. 2019). Many of the managed EAZA breeding programs are largely unsustainable as well (Leus et al., 2011). For many species, periods of non-breeding are negatively associated with reproductive success (Asa et al., 2014; Penfold et al., 2014). For tigers specifically, the age and reproductive history of the female are the primary indicators of litter production; the probability of a female producing a litter declines after 5-6 years of age and is substantially lower for nulliparous females (Saunders, et al., 2014). Management programs for tigers should therefore focus on retaining fertility by breeding nulliparous females before they are 5 years of age (Penfold, et al. 2014). As pregnancy is also suggested to have a protective function against infertility and uterine pathology in carnivores (Penfold, et al., 2014), allowing a female to reproduce once, at a young age, and then using contraceptives later, may be an effective strategy to preserve reproductive capacity.

**Limitations**

There were several limitations to this project including poor survey response rate and the reliance upon retrospective self-reported, often incomplete, records. The quality of record keeping was variable between institutions and important pieces of data, such as implant site placement, drug doses and specific products used, dates of mate access, weight gain and observations of behavioral estrus signs were sometimes missing or incomplete. Rates of behavioral reversal reported here may, for example, not fully reflect rates in the study populations as overt estrus behaviors in tigers including lordosis, rolling and solicitation, male interest, and breeding attempts (Putman et al., 2015), were rarely recorded by participating institutions. Signs of estrus might be easily missed as many exotic felids experience ‘silent estrus,’ during which there may be no obvious signs of estrus (Putman et al., 2015), particularly if a female does not have access to a mate. Lastly, pathologies may be underreported as the database is focused on reproductive data and collections may not report non-reproductive pathologies. This study cannot discern whether the incidence of reproductive pathologies in contracepted tigers differs from that in uncontracepted tigers or tigers separated from mates in the analysis presented here. The lack of association between variables such as implant removal and full or partial reversals in this study may also, in part, be because of the small sample size available for analysis, further highlighting the need for more complete and comprehensive data collection across zoological institutions.

**CONCLUSIONS AND RECOMMENDATIONS:**

This study determined that the current use of DA in tigers is variable with respect to dose, frequency, and site placement. Incorrect protocols were commonly used, particularly pertaining to supplementary use of MA upon the first bout of DA treatment, the lack of implant removal, and the preference for manufacturer’s recommended location sites, highlighting that current recommendations are poorly understood or not implemented consistently. This study demonstrates that, in general, institutions are replacing deslorelin implants according to the manufacturer’s guidelines (six months for 4.7 mg and 12 months for 9.4 mg). This study demonstrates that contraception with DA in tigers is effective, reversible, and is associated with fewer health concerns than previously commonly used contraceptives. This study did not identify any significant pathology associated with DA, to date, which is a substantial improvement over synthetic progestin contraceptives.

Further studies should investigate the current individual and population level impacts of contraceptive use, especially DA, including a more in-depth pathology review, especially in female tigers. Future record keeping must be thorough and detailed; current records are incomplete, making data evaluation difficult. Professional organizations should be certain that all institutions understand and use the latest contraceptive protocol recommendations and institutions should be required to collect and report behavioral and hormonal data.

**ACKNOWLEDGMENTS**

The authors would like to thank the following people and organizations for their support of and contributions to this project: The Zoological Society of London, Malcolm Fitzpatrick, Teague Stubbington, Jo Cook, the tiger EEP, John Lewis, MA, VetMB, PhD, Dipl. ECZM, MRCVS and the institutions that contributed records to the Contraception Database.

**REFERENCES**

1. Ackerman, C.L., Volpato, R., Destro, F.C., Trevisol, E., Ruas Sousa, N., Guaitolini, C.R., Derussi, A.A., Rascado, T.S., Lopes, M.D. (2012). Ovarian activity reversibility after the use of deslorelin acetate as a short-term contraceptive in domestic queens. *Theriogenology*, 78, 817-822.
2. Asa, C.S. (2005a). Types of contraception: the choices. In C.S. Asa and I.J. Porton (Eds.), *Wildlife Contraception: Issues, Methods, and Applications* (pp. 29-52). Baltimore, Maryland: The Johns Hopkins University Press.
3. Asa, C.S., Porton, I.J., Calle, P.P. (2005c). Choosing the most appropriate contraceptive. In C.S. Asa and I.J. Porton (Eds.), *Wildlife Contraception: Issues, Methods, and Applications* (pp. 83-95). Baltimore, Maryland: The Johns Hopkins University Press.
4. Asa, C., Boutelle, S., Bauman, K. (2012). AZA wildlife contraception center programme for wild felids and canids. *Reproduction of Domestic Animals*, 47, (Suppl. 6), 377-380.
5. Asa, CS, Bauman, KL, Devery, S, Zordan, M, Camilo, GR, Boutelle, S, Moresco, A. (2014) Factors Associated with Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility. *Zoo Biology* 33: 8-19.
6. Bates, D., Machler, M., Bolker, B., Walker, S. (2015). Fitted Linear Mixed-Effects Models Using Ime4. *Journal of Statistical Software*, 67(1). http://doi.org/10.18637/jss.v067.i01
7. Bertschinger, H.J., Asa, C.S., Calle, P.P., Long, J.A., Bauman, K., DeMatteo, K., Jochle, W., Trigg, T.E., Human, A. (2001). Control of reproduction and sex related behavior in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. *Journal of Reproduction and Fertility Supplement*, 57, 275-283.
8. Bertschinger, H.J., de Barros Vaz Guimaraes, M.A., Trigg, T.E., Human, A. (2008). The use of deslorelin implants for the long-term contraception of lionesses and tigers. *Wildlife Research*, 35, 525-530.
9. Bertschinger, H.J. and Sills, E.S. (2013). Contraceptive applications of GnRH-analogs and vaccines for wildlife mammals of southern Africa: current experience and future challenges. In S. Sills (Ed.) *Gonadotropin-Releasing hormone (GnRH). Production, structure and function* (pp. 85-108). New York: Nova Science Publications Inc.
10. Brown, J.L. (2011). Female reproductive cycles of wild female felids. *Animal Reproduction Science*, 124, 155-162.
11. Brown, J.L. (2018). Comparative ovarian function and reproductive monitoring of endangered mammals. *Theriogenology*, 2018, 109, 2-13.
12. Cabot, M., Ramsay, E. Chaffins, D., Sula, M. (2020). Histologic evidence of spontaneous ovulation in tigers (*Panthera tigris*). *Journal of Zoo and Wildlife Medicine*, 51, 652-656.
13. Cecchetto, M, Salata, P., Baldan, A., Milani, C., Mollo, A., Fontaine, C., Sontas, H., Gelli, D., De Benedictis, G., Stelletta, C., Romagnoli, S. (2017). Postponement of puberty in queens treated with deslorelin. *Journal of Feline Medicine and Surgery*, 19, 1224-1230.
14. Che-Castaldo J, Johnson, B., Magrisso, N., Mechak, L., Melton, K., Mucha, K., Terwilliger, L., Theis, M., Long, S., Faust, L. (2019) Patterns in the long-term viability of North American zoo populations. *Zoo Biology*, 38, 78-94. DOI: 10.1002/zoo.21471
15. Chuei, J.Y., Asa, C.S., Hall-Woods, M., Ballou, J., Traylor-Holzer, K. (2007). Restoration of reproductive potential after expiration or removal of melengestrol acetate contraceptive implants in tigers (*Panthera tigris*). *Zoo Biology*, 26, 275-288.
16. Cook, J. (2019): EAZA studbook for Amur tigers,*Panthera tigris altaica*. Zoological Society of London. Retrieved from www.eaza.net
17. Council Directive 88/146/EEC of 7 March 1988 prohibiting the use in livestock farming of certain substances having a hormonal action. (2020, July 30). Retrieved from <https://eur-lex.europa.eu/eli/dir/1988/146/oj>
18. Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of ß-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. (2020, July 30) Retrieved from <https://eur-lex.europa.eu/eli/dir/1996/22/oj>
19. Cowl, V.B., Walker, S.L., Rambaud, Y.F. (2018). Assessing the efficacy of deslorelin acetate implants (Suprelorin®) in alternative placement sites. *Journal of Zoo and Wildlife Medicine*, 49, 1-8.
20. Dematteo, K.E. (2005). Contraception in carnivores. In C.S. Asa and I.J. Porton (Eds.), *Wildlife Contraception: Issues, Methods, and Applications* (pp. 105-118). Baltimore, Maryland: The Johns Hopkins University Press.
21. EGZAC: EAZA Group on Zoo Animal Contraception. (2020, July 30). Retrieved from <http://www.egzac.org/>
22. European Medicines Agency: Suprelorin® (deslorelin acetate). (2020, July 30). Retrieved from <https://www.ema.europa.eu/en/medicines/veterinary/EPAR/suprelorin>
23. Felidae: Carnivore. (2020, July 30). Retrieved from: <https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/contraceptionrecommendatio/contraceptionmethods/felidae>
24. Fontaine, C. (2015). Long-term contraception in a small implant; a review of Suprelorin® (deslorelin) studies in cats. *Journal of Feline Medicine and Surgery*, 17, 766-771.
25. Franklin, AD, McDonald, MM, Powell, DM. You Help Me, I Help You: Data Collection for Informed Use of Contraception. In: Proceedings of the 38th Annual Conference of Zoo Veterinary Technicians; 2018. p. 30-32.
26. GnRH agonist-Deslorelin (Suprelorin®). (2020, July 31). Retrieved from https://www.egzac.org/home/viewdocument?filename=Deslorelin.pdf
27. Goericke-Pesch, S. (2017). Long-term effects of GnRH agonists on fertility and behavior. *Reproduction of Domestic Animals*, 52, (Suppl. 2), 336-347.
28. Goodrich, J. Lynam, A., Miquelle, D., Wibisono, H., Kawanishi, K., Pattanavibool, A., Htun, S., Tempa, T., Karki, J., Jhala, Y., Karanth, U., (2015). *Panthera tigris*, The IUCN Red List of Threatened Species 2015. e.T15955A50659951. <http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T15955A50659951.en>. [Downloaded on 16 December 2019].Downl
29. Graham, L.H., Byers, A.P., Armstrong, D.L., Loskutoff, N.M., Swanson, W.F., Wildt, D.E., Brown, J.L. (2006). Natural and gonadotropin-induced ovarian activity in tigers (*Panthera tigris*) assessed by fecal steroid analyses. *General and Comparative Endocrinology*, 147, 362-370.
30. Graham, L.H., Goodrowe, K.L., Raeside, J.I., Liptrap, R.M. (1995). Non-invasive monitoring of ovarian function in several felid species by measurement of fecal estradiol-17β and progestins. *Zoo Biology*, 14, 223-237.
31. Gray, M.E., Cameron, E.Z. (2010). Does contraceptive treatment in wildlife result in side effects? A review of quantitative and anecdotal evidence. *Reproduction*, 139, 45-55.
32. Gu, J., Guo, Y., Stott, P., Jiang, G., Jianzhang, M.A. (2016). A comparison of reproductive parameters of female Amur tigers (*Panthera tigris altaica*) in the wild and captivity. *Integrative Zoology*, 11, 33-39.
33. Harrenstien, L.A., Munson, L., Seal, U.S. (1996). Mammary cancer in wild felids and risk factors for its development: a retrospective study of the clinical behavior of 31 cases. *Journal of Zoo and Wildlife Medicine*, 27, 468-476.
34. Holt, V.L, Cushing-Haugen, K.L., Daling, J.R. (2002) Body weight and risk of oral contraceptive failure. *Obstetrics & Gynecology*, 99, 820-7. DOI: 10.1016/s0029-7844(02)01939-7
35. Junaidi, A., Williamson, P.E., Cummins, J.M., Martin, G.B., Blackberry, M.A., Trigg, T.E. (2003). Use of a new drug delivery formulation of the gonadotrophin-releasing hormone analogue Deslorelin for reversible long-term contraception in male dogs. *Reproduction, Fertility and Development*, 15, 317-322.
36. Kapp, N., Abitbol, J.L., Mathe, H, Scherrer, B., Guillard, H., Gainer, E., Ulmann, A., (2015). Effect of body weight and BMI on the efficacy of levonorgestrel emergency contraception. *Contraception*, 91, 97-104. DOI: 10.1016/j.contraception.2014.11.001
37. Kazensky, C.A., n, L., Seal, U.S. (1998). The effects of melengestrol acetate on the ovaries of captive wild felids. *Journal of Zoo and Wildlife Medicine*, 29, 1-5.
38. Larsen J.A. (2017). Risk of obesity in the neutered cat. *Journal of Feline Medicine and Surgery*, 19, 779-783.
39. Leus, K., Bingaman Lackey, L., van Lint, W. de Man, D., Riewalk, S., Veldkam, A., & Wijmans, J. (2011). Sustainability of European Association of Zoos and Aquaria bird and mammal populations. *WAZA Magazine*, *12*, 11-14.
40. Lewis, J.C. (2016). Sumatran tiger (*Panthera tigris sumatrae*) EEP Status and Recommendations 2017, Appendix V, Tiger EEP: Contraception. 56-61.
41. McAloose, D., Munson, L., Naydan, D.K. (2007). Histologic features of mammary carcinomas in zoo felids treated with melengestrol acetate (MGA) contraceptives. *Veterinary Pathology*, 44, 320-326.
42. McEvoy, O.K., Miller, S.M., Beets, W., Bodasing, T., Borrego, N., Burger, A., Courtenay, B., Ferreira, S., Hanekom, C., Hofmeyr, M., Packer, C., Robertson, D., Stratford, K., Slotow, R., Parker, D.M. (2019). The use of contraceptive techniques in managed wild African lion (*Panthera leo*) populations to mimic open system cub recruitment. *Wildlife Research*, https://doi.org/10.1071/WR18079
43. Moresco, A., Agnew, D.W. (2013). Reproductive health surveillance in zoo and wildlife medicine. *Journal of Zoo and Wildlife Medicine*, 44, S26-S33.
44. Moresco, A., Dadone, L., Arble, J., Klaphake, E., Agnew, D.W. (2014). Location and removal of deslorelin acetate implants in female African lions (*Panthera leo*). *Journal of Zoo and Wildlife Medicine*, 45, 397-401.
45. Munson, L. (2006). Contraception in felids. Theriogenology, 66, 126-134.
46. Munson, L., Gardner, I.A., Mason, R.J., Chassy, L.M., Seal, U.S. (2002). Endometrial hyperplasia and mineralization in zoo felids treated with melengestrol acetate contraceptives. *Veterinary Pathology*, 39, 419-427.
47. Nguyen, P.G., Dumon, H.J., Siliart, B.S., Martin, L.J., Sergheraert, R., Biourge, V.C. (2004). Effects of dietary fat and energy on body weight and composition after gonadectomy in cats. *American Journal of Veterinary Research*, 65, 1708-1713.
48. Penfold, L.M., Powell, D., Traylor-Holzer, K., Asa, C.S. (2014). ‘Use it or Lose it’: Characterization, Implications, and Mitigation of female infertility in captive wildlife. *Zoo Biology*, 33, 20-28.
49. Powell, D.M., Asa, C.S. (2019). Reality Check: Complications and complexities involved with contraception and assisted reproductive technology application in wildlife. *Animal Keepers’ Forum*, 46(7):180-185.
50. Putman, S.B., Brown, J.L., Franklin, A.D., Schneider, E.C., Boisseau, N.P., Asa, C.S., Pukazhenthi, B.S. (2015). Characterization of ovarian steroid patterns in female African lions (*Panthera leo*), and the effects of contraception on reproductive function. *PLos ONE*, 10(10): e0140373
51. R Development Core Team, 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. (2020, July 30). Retrieved from http://www.r-project.org/
52. Sadleir, R.M. (1966). Notes on reproduction in the larger Felidae. *International Zoo Yearbook*, 6(1), 184-187.
53. Saunders, S.P., Harris, T., Traylor-Holzer, K., Beck, K.G. (2014). Factors influencing breeding success, ovarian cyclicity, and cub survival in zoo-managed tigers (*Panthera* *tigris*). *Animal Reproduction Science*, 144, 38-47.
54. Seal, U.S., Barton, R., Mather, L., Olberding, K., Plotka, E.D., Gray, C.W. (1976). Hormonal contraception in captive female lions (*Panthera leo*). *Journal of Zoo Animal Medicine*, 7, 12-20.
55. Strike, T. (2020, July 30) EAZA RMC Felid Guidelines (2014). Retrieved from <https://www.egzac.org/home/viewdocument?filename=Felidae.pdf.pdf>
56. Stubbington, T. Fitzpatrick, M. Cook, J. (2019): EAZA Regional studbook for Sumatran tigers, *Panthera tigris sumatrae*. Zoological Society of London. Retrieved from www.eaza.net
57. Traylor-Holzer, K. (2019a). AZA Amur tiger regional studbook. Association of Zoos & Aquariums. Retrieved from [www.aza.org](http://www.aza.org)
58. Traylor-Holzer, K. (2019b). AZA Malayan tiger regional studbook. Association of Zoos & Aquariums. Retrieved from [www.aza.org](http://www.aza.org)
59. Traylor-Holzer, K. (2019c). AZA Sumatran tiger regional studbook. Association of Zoos & Aquariums. Retrieved from [www.aza.org](http://www.aza.org)
60. Wildt D, Swanson W, Brown J, Sliwa A, Vargas A. 2010. Felids *ex situ*: managed programmes, research, and species recovery. In: Biology and Conservation of Wild Felids. 217-235. D.W. MacDonald and A.J. Loveridge, editors. Oxford University Press, New York, NY.