

Jaguar Veterinary Guidelines Contraception

Separation of the sexes:

Separation of the sexes is not recommended for carnivores due to the increased risk of reproductive failure and uterine pathology in cheetahs (Crosier et al. 2011) and canids (Asa et al. 2014). Similar research has not been conducted in jaguars, but the effects of separation may be similar.

Surgical sterilization:

Males:

Castration (recommended):

Permanent sterilisation by surgical gonadectomy. This procedure should not be carried out in individuals who are likely to receive breeding recommendations. As the testes are removed, testosterone mediated aggression may decrease. Males may also experience a decrease in body size and testicle size. If pre-pubescent males are castrated, they may become taller due to the delay in the closure of the epiphyseal plates.

Vasectomy (not recommended):

Vasectomies are not recommended in this species due to the risk of females developing reproductive pathologies as a result of repeated oestrus cycles and induced ovulations through mating but without conceiving.

Females:

Ovariohysterectomy:

For individual jaguars unlikely to receive a breeding recommendation, permanent sterilisation by surgical ovariohysterectomy for females. Removal of the uterus as well as ovaries is preferable in older females, due to the increased likelihood of uterine pathology with age.

Ovariectomy:

Removal of ovaries is a safe and effective method to prevent reproduction for animals that are eligible for permanent sterilization. In general, ovariectomy is sufficient in young females.

Tubal ligation (not recommended):

Tubal ligation will not prevent the potential adverse effects to females that can result from prolonged, cyclic exposure to the endogenous progesterone associated with the pseudo-pregnancy that follows ovulation induced by copulation.

Hormonal contraception:

Males and females:

GnRH agonists (recommended):

GnRH agonists, such as deslorelin (Suprelorin; guideline below) or leuprolide acetate (Lupron), reversibly suppress the reproductive endocrine system, preventing production of pituitary hormones



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(follicle stimulating hormones, luteinizing hormone) and gonadal hormones (estradiol and progesterone in females, testosterone in males). The observed effects are similar to those following either ovariectomy in females or castration in males, but are reversible. GnRH agonists first stimulate the reproductive system, which can result in oestrus and ovulation in females or temporary enhancement of testosterone and semen production in males. The stimulation phase must be suppressed in females to prevent the sustained elevation of progesterone following ovulation by treatment with an oral progestogen (Megestrol acetate; Megace[®]/Ovaban[®]/Ovarid[®]) for 7 days before and 8 days after implant insertion (Wright et al. 2001). The risk of developing uterine pathologies following the 15 day treatment is much reduced compared to the 3-4 week stimulation phase. The stimulation phase cannot be suppressed in males.

Deslorelin acetate (Suprelorin[®] - subcutaneous implant):

In males, 1 x 4.7mg implants are recommended for a minimum duration of 6 months and 1 x 9.4mg are recommended for a minimum duration of one year. In females, 2 x 4.7mg implants are recommended for a minimum duration of 6 months and 2 x 9.4mg are recommended for a minimum duration of 6 months and 2 x 9.4mg are recommended for a minimum duration of one year. Due to the initial stimulation of the reproductive system, the first bout in females must also be supplemented with additional contraception e.g. oral megestrol acetate (Ovarid[®]/Megace[®]) daily 7 days before and 8 days after placing the implants. In males, the stimulation period cannot be suppressed therefore additional contraception should be used in females or the sexes should be separated for 6-8 weeks after implant placement, to allow for viable sperm to clear from his system. Suprelorin is designed to be fully reversible although time to reversal is variable in felids. In order to increase the chances of a full reversal, place the implant in such a way that facilitates removal e.g. in the umbilical region, inner thigh, armpit.

Suprelorin should not be used during pregnancy, since it may cause spontaneous abortion or prevent mammary development necessary for lactation. It may prevent initiation of lactation by inhibiting progesterone secretion, but effects on established lactation are less likely. New data from domestic cats have shown no effect on subsequent reproduction when treatment began before puberty.

Suprelorin is more commonly used in females, because monitoring efficacy in females by suppression of oestrous behaviour or gonadal steroids in faeces is easier than ensuring continued absence of sperm in males.

Females only:

Progestin-based contraceptives (e.g. Implanon[®]/Nexplanon[®], Depo-Provera[®], Delvosteron[®], Jadelle[®]):

Progestin-based contraceptives are not recommended for use in female jaguars due to the increased risk of mammary and uterine pathologies of elevated levels of circulating progestogens without a resulting pregnancy.



Assisted reproductive techniques:

Reproductive hormone monitoring:

Reproductive hormones can non-invasively be monitored through faecal sampling. For males, faecal samples should be collected 1-3 times per week, while faecal samples should be collected 3-4 times per week for females.

In wild Jaguar males, faecal androgens are significantly higher in the wet season than in the dry season (Morato et al. 2001).

In Jaguar females, behavioural oestrus can be characterised by rolling, disinterest in food, vulvar swelling, discharge, and lordosis (Barnes et al. 2016). Hormonal oestrus in Jaguar females was classified when estradiol concentrations were greater than the baseline + 1 standard deviation (SD) in the study by Barnes et al. (2016). Baseline concentrations are calculated by using an iterative process in which values that exceed the mean + 1.5 SD are excluded until no values exceed the mean + 1.5 SD (Brown et al. 2001). Hormonal oestrus lasts for 6.5 ± 0.3 days (mean \pm SD), while behavioural oestrus lasts for 7.8 ± 0.7 days (Barnes et al. 2016). Pregnancy and pseudopregnancy can be characterized by monitoring faecal progesterone concentrations. However, it may be difficult to discriminate between conception and non-conceptive luteal phases (pseudopregnancy) by progesterone only. Baseline concentrations are calculated as for estradiol. Luteal progesterone concentrations are classified when progesterone concentrations return to baseline following a sustained rise of > 20 days (Barnes et al. 2016). Pregnancy lasts approximately 98 days in jaguars (Figure 1).



Figure 1. Endocrine profile for a pregnant jaguar following a natural mating. Figure taken from Barnes et al. (2016).

Understanding ovarian activity is essential for the successful application of assisted reproductive techniques (Pelican et al. 2006).



Fertility evaluation and gamete recovery: Males:

Semen collection under anaesthesia is an accurate method of gaining information on male fertility. Each testis should be measured and a combined testicular volume should be calculated (Howard 1993). Reference values for captive and wild jaguars can be found in Morato et al. (2001). The penis should be extruded from its sheath and examined for the presence of spines (scale of 1-3, 3 = most prominent spines (Morato et al. 2001; Swanson et al. 1995). Sperm can be collected by electro ejaculation (Morato et al. 1999, 2001) or urethral catheterization (Ribeiro de Araujo et al. 2018) and should be evaluated by measuring: 1) semen volume, semen concentration (sperm count), total motility and forward progressive motility; 2) morphology: proportions of normal and abnormal sperm forms (Figure 2), all via phase microscopy (630x) (Howard et al. 1990); and 3) acrosomal integrity using specific stains, such as the rose bengal/fast green stain (Pope et al. 1991). Reference values for captive and wild jaguars can be found in Morato et al. (2001). Semen is usually very dilute (~5 x 10⁶/ml) but large volumes are generally recovered (up to 20 ml) (Morato et al. 2001; Swanson et al. 1995). A high percentage of abnormal sperm morphology is common in feline species, and in jaguars reported to be up to 65% in captive jaguars and 51% in wild jaguars (Morato et al. 2001).

Fine needle aspiration of the testes/epidymidiscan be used to evaluate sperm production if semen collection is not possible (Leme et al. 2018).

Semen cryopreservation has been performed successfully in various feline species, one applicable method is described by Swanson *et al.* (2003). Reported survival following cryopreservation is relatively good (Swanson et al. 1996), with sperm retaining 25-50% motility post-thaw (Graham et al. 1978), depending on initial quality of the sample.



Figure 2. Morphology of jaguar spermatozoa. a) normal morphology, b) malformed head shape, c) biflagellate, d) tightly coiled flagellum. Figure taken from Morato et al. (2001).



Females:

Hormonal monitoring is encouraged to determine whether females are reproductively active.

From live animals, the successful recovery of oocytes has been described in Morato et al. (2000).

From deceased animals, the collection of oocytes can be accomplished 8 hours after death ovary dissection and mechanical follicle isolation (Jewgenow et al. 1997). Preantral follicles can also be collected from deceased animals, following methods in Jewgenow and Stolte (1996) or Jewgenow *et al* (1997), and matured *in vitro*.

Ovarian stimulation:

The success of assisted reproductive techniques (ARTs) such as *in vitro* fertilization (IVF) or artificial insemination (AI) in felids is generally low. Oestrus induction via exogenous hormones is desired to have timed and planned procedures and several protocols for felids are published (Brown et al. 1995; Goeritz et al. 2012; Howard et al. 1992; Kutzler 2007; Pelican et al. 2006), however, the overall success rate of these protocols is limited. Since jaguars express overt signs of heat, natural oestrus AI may be a promising option.

Ovulation induction:

In jaguars, ovulation during natural heat can be induced through using exogenous gonadotropins (protocols described in Barnes et al. 2016), but can in some cases be induced by physical vaginal stimulation (Barnes et al. 2016). Ovulation in exotic felids tends to occur approximately 37-42 hours after treatment with gonadotropins (Pelican et al. 2006). The most common exogenous hormone treatment for ovulation induction reported is human Choriogonadotropin (hCG) or Gonadotropin Releasing Hormone (GnRH) (Callealta et al. 2019).

Artificial insemination (AI):

Anecdotally, successful AI has been achieved in jaguars however; exact techniques have not been published. Successful AI protocols have been published for a number of feline species. One should distinguish between non-surgical and surgical AI options. Successful, non-surgical AI during natural heat has been repeatedly performed in Asiatic golden cats, Persian leopards and African lion (Callealta et al. 2019; Dresser et al. 1982; Lueders et al. 2014, 2015).

The most commonly reported AI method for smaller felids is the laparoscopic approach, where the semen sample is placed into the oviduct or uterine horn under endoscopic abdominal view.

In vitro fertilization:

Techniques have been described in Morato et al. (2000).

References

- Asa, C. S., Bauman, K. ., Devery, S., Zordan, M., Camilo, G. R., Boutelle, S., & Moresco, A. (2014). Factors Associated with Uterine Endometrial Hyperplasia and Pyometra in Wild Canids: Implications for Fertility. *Zoo Biology*, *33*(1), 8–19.
- Barnes, S. A., Teare, A. J., Staaden, S., Metrione, L., & Penfold, L. M. (2016). Characterization and manipulation of reproductive cycles in the jaguar (Panthera onca). *General and Comparative Endocrinology*, 225, 95–103. doi:10.1016/j.ygcen.2015.09.012

Brown, J. L., Graham, L. H., Wielebnowski, N., Swanson, W. F., Wildt, D. E., & Howard, J. G. (2001).



Understanding the basic reproductive biology of wild felids by monitoring of faecal steroids. *Journal of reproduction and fertility. Supplement*, *57*, 71–82. http://www.ncbi.nlm.nih.gov/pubmed/11787193. Accessed 5 November 2019

- Brown, J. L., Wildt, D. E., Graham, L. H., Byers, A. P., Collins, L., Barrett, S., & Howard, J. G. (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. doi:https://doi.org/10.1095/biolreprod53.1.93
- Callealta, I., Ganswindt, A., Malan, M., & Lueders, I. (2019). Non-surgical artificial insemination using a GnRH analogue for ovulation induction during natural oestrus in African lions (Panthera leo). *Theriogenology*, *139*, 28–35.
- Crosier, A. E., Comizzoli, P., Baker, T., Davidson, A., Munson, L., Howard, J. G., et al. (2011). Increasing Age Influences Uterine Integrity, But Not Ovarian Function or Oocyte Quality, in the Cheetah (Acinonyx jubatus)1. *Biology of Reproduction*, *85*(2), 243–253. doi:10.1095/biolreprod.110.089417
- Dresser, B. L., Kramer, L., Reece, B., & Russell, P. T. (1982). Induction of ovulation and successful artificial insemination in a Persian leopard (Panthera pardus saxicolor). *Zoo Biology*, 1(1), 55–57. doi:10.1002/zoo.1430010106
- Goeritz, F., Painer, J., Jewgenow, K., Hermes, R., Rasmussen, K. L. R., Dehnhard, M., & Hildebrandt, T.
 B. (2012). Embryo Retrieval after Hormonal Treatment to Control Ovarian Function and Nonsurgical Artificial Insemination in African Lions (Panthera leo). *Reproduction in Domestic Animals*, 47(s6). doi:https://doi.org/10.1111/rda.12026
- Graham, E. F., Schmehi, M. K. L., Evensen, B. K., & Nelson, D. . (1978). Semen preservation in nondomestic mammals. In *Symposium of the Zoological Society London* (pp. 153–173). London.
- Howard, J. G. (1993). Semen collection and analysis in carnivores. In M. E. Fowler (Ed.), *Zoo and wild animal medicine: current therapy* (3rd ed., pp. 390–399). Philadelphia: W.B. Saunders Co.
- Howard, J. G., Brown, J. L., Mitchell, B., & Wildt, D. E. (1990). Teratospermic and Normospermic Domestic Cats: Ejaculate Traits, Pituitary—Gonadal Hormones, and Improvement of Spermatozoal Motility and Morphology After Swim-Up Processing. *Journal of Andrology*, 11(3), 204–215. doi:10.1002/j.1939-4640.1990.tb03229.x
- Howard, J. G., Donoghue, A. M., Barone, M. A., Goodrowe, K. L., Blumer, E. S., Snodgrass, K., et al. (1992). Successful Induction of Ovarian Activity and Laparoscopic Intrauterine Artificial Insemination in the Cheetah (Acinonyx jubatus). *Journal of Zoo and Wildlife Medicine*, 23(3), 288–300.
- Jewgenow, K., Blottner, S., Lengwinat, T., & Meyer, H. H. (1997). New methods for gamete rescue from gonads of nondomestic felids. *Journal of Reproduction and Fertility Supplement*, *51*, 33–39.
- Jewgenow, K., & Stolte, M. (1996). Isolation of preantral follicles from nondomestic cats Viability and ultrastructural investigations. *Animal Reproduction Science*, *44*(3), 183–193. doi:10.1016/0378-4320(96)01549-7
- Kutzler, M. A. (2007). Estrus induction and synchronization in canids and felids. *Theriogenology*, *68*(3), 354–374. doi:10.1016/j.theriogenology.2007.04.014
- Leme, D. P., Visacre, E., Castro, V. B., & Lopes, M. D. (2018). Testicular cytology by fine needle aspiration in domestic cats. *Theriogenology*, *106*, 46–52.



doi:10.1016/j.theriogenology.2017.10.012

- Lueders, I., Ludwig, C., Schroeder, M., Mueller, K., Zahmel, J., & Dehnhard, M. (2014). Successful nonsurgical artificial insemination and hormonal monitoring in an Asiatic golden cat (Catopuma temmincki). *Journal of Zoo and Wildlife Medicine*, *45*(2), 372–379.
- Lueders, I., Ludwig, H., & Weber, C. (2015). Nonsurgical artificial insemination in felids: Asiatic golden cat (Catopuma temmincki) and Persian leopard (Panthera pardus saxicolor). In *International Conference on Diseases of Zoo and Wild Animals* (p. 59). Barcelona.
- Morato, R. G., Conforti, V. A., Azevedo, F. C., Jacomo, A. T., Silveira, L., Sana, D., et al. (2001). Comparative analyses of semen and endocrine characteristics of free-living versus captive jaguars (Panthera onca). *Reproduction*, *122*, 745–751.
- Morato, R. G., Crichton, E. G., Paz, R. C. R., Zuge, R. M., Maura, C. A., & Nunes, A. L. V. (2000). Ovarian stimulation and successful in vitro fertilization in the jaguar (Panthera onca). *Theriogenology*, *53*, 399.
- Pelican, K. M., Wildt, D. E., Pukazhenthi, B., & Howard, J. G. (2006). Ovarian control for assisted reproduction in the domestic cat and wild felids. *Theriogenology*, *66*(1), 37–48. doi:10.1016/j.theriogenology.2006.03.013
- Pope, C. E., Zhang, Y. Z., & Dresser, B. L. (1991). A simple staining method for quantifying the acrosomal status of cat spermatozoa. *Journal of Zoo and Wildlife Medicine*, 22(1), 97–95. doi:10.1016/0093-691X(91)90233-4
- Ribeiro de Araujo, G., Rego de Paula, T. A., de Deco-Souza, T., Morato, R. G., Bergo, L. C. F., Cruz da Silva, L., et al. (2018). Comparison of semen samples collected from wild and captive jaguars (Panthera onca) by urethral catheterization after pharmacological induction. *Animal Reproduction Science*, *195*, 1–7. doi:10.1016/j.anireprosci.2017.12.019
- Swanson, W. F., Johnson, W. E., Cambre, R. C., Citino, S. B., Quigley, K. B., Brousset, D. M., et al. (2003). Reproductive status of endemic felid species in latin american zoos and implications for Ex situ conservation. *Zoo Biology*, *22*(5), 421–441. doi:10.1002/zoo.10093
- Swanson, W. F., Roth, T. L., Blumer, E., Citino, S. B., Kenny, D., & Wildt, D. E. (1996). Comparative cryopreservation and functionality of spermatozoa from the normospermic jaguar (Panthera onca) and teratospermic cheetah (Acinonyx jubatus). *Theriogenology*, 45(1), 241. doi:10.1016/0093-691x(96)84714-5
- Swanson, W. F., Wildt, D. E., Cambre, R. C., Citino, S. B., Quigley, K. B., Brousset, D., et al. (1995). Reproductive survey of endemic felid species in Latin American zoos: male reproductive status and implications for conservation. In *Proceedings of the American Association of Zoo Veterinarians Annual Meeting* (pp. 374–380). Houston: AAZV.
- Wright, P. J., Verstegen, J. P., Onclin, K., Jochle, W., Armour, A. F., Martin, G. B., & Trigg, T. E. (2001).
 Suppression of the Oestrus Responses of Bitches to the GnRH Analogue Deslorelin by Progestin.
 Journal of Reproduction and Fertility. Supplement, 57, 263–283.