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Reproductive aspects of the female jaguar (*Panthera onca* L.)

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ABSTRACT

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Objective: To describe the reproductive characteristics of female jaguars and assisted reproduction techniques.

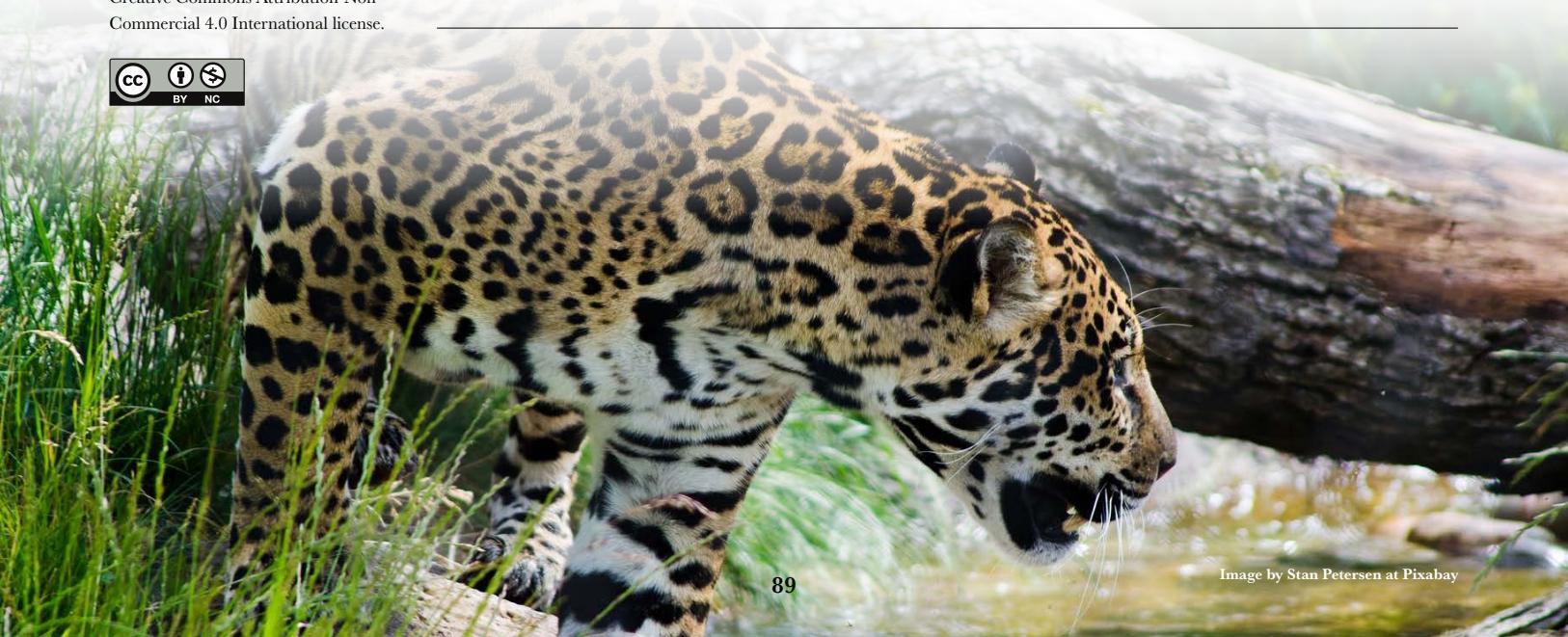
Methodology: A literature review about reproductive characteristics and assisted reproduction techniques was done to document information on this subject.

Results: Jaguars are the largest feline native to America; females sizes range from 1.57 to 2.19 m in length and weigh 45 to 82 kg. Jaguar females are ready for mating at about 2 years old and have an estrous cycle every 37 days. The reproductive behaviour patterns of the jaguar in captivity are grouped into four categories: 1) sexual, 2) affiliative, 3) agonists and 4) individual. The gestation period lasts between 91 to 111 days, and usually give birth to one to four young. For assisted reproduction, artificial insemination contributes to the genetic distribution or variability of germplasm.

Limitations on study: The destruction of forests and jungles, habitat fragmentation, indiscriminate hunting, the lack of prey and food sources and the conflict with ranchers put Jaguars on the brink of extinction. Also, there is few information about their reproductive characteristics, which is why in Mexico, it is imperative to generate this information, mainly for free-living jaguars.

Conclusions: The reproductive characteristics and assisted reproductive techniques in the female jaguar were documented. However, work is needed on assisted reproduction techniques in this species, which will contribute to the conservation of oocytes or embryos. It is advisable to design protocols to control and induce ovulation in the female *Panthera onca*.

Keywords: reproduction; feline; female; preservation.



DESCRIPTION

The jaguar is the largest feline in the Americas (Emmons, 1987; Chávez *et al.*, 2005). Its size varies geographically. Brazil is the country where the largest specimens have been found, with a body length, without the tail, up to 1.85 m (Nowak and Paradiso, 1983); its body is robust, with short and muscled forelimbs and hind limbs. Females measure between 1.57 and 2.19 m length and weigh 45 to 82 kg (Leopold, 1965; Seymour, 1989). The skin coloration varies from pale yellow to reddish brown, the belly color and inner part of the legs is pale, almost white or totally white. To the naked eye spots can be observed called rosettes on the body, of variable size, which have small spots in the center (Yescas and Ramírez, 2013). There is another phenotype with black or blackish-brown fur in which the rosettes are barely noticeable (Hoogesteijn and Mondolfi, 1993; Chávez *et al.*, 2005). Historically, it had a wide distribution, from southern United States to Argentina (Hall, 1981; Ceballos and Oliva, 2005). In Mexico, it distributes in tropical and subtropical regions, from the United States border, through Sonora and Tamaulipas to Chiapas and the Yucatan Peninsula (Figure 1; CONANP, 2009; Ceballos *et al.*, 2011; Yescas and Ramírez, 2013).

REPRODUCTION

Reproductive tract anatomy

The structures that form the female reproductive system are: ovaries, oviducts, uterus, vagina and vulva (Figure 2). The ovaries are surrounded by the ovarian pouch formed by



Figure 1. Current jaguar distribution according to CONABIO records (2010). Taken from <http://www.conabio.gob.mx/informacion/gis/layouts/panoncadcgw.png>. Consultation of use of license at <https://creativecommons.org/licenses/by-nc/2.5/mx/legalcode>

the mesosalpinx and mesovarium. As in other carnivore species, the ovaries (Figure 2) are located caudal to the kidneys, in a dorsal position into the abdominal cavity (Mayor and Lopez, 2010). The oviducts are tubular structures of sinuous trajectory included in the mesosalpinx, with considerable peristaltic activity during estrus. The oviducts transport oocytes to the uterine horns for their subsequent implantation in the uterus (Barrueta, 2012). The uterus is bicornuate and gestation can take place in both horns, the body of the uterus is reduced, the cervix has a thick muscular wall that isolates the uterus from the outside. The vagina is longer than the vaginal vestibule, shared by the genital and urinary tracts. The vulva is formed by two lips that join at the dorsal and ventral vulvar commissures. The clitoris has a rudimentary glans and is centrally located within the clitoral fossa (Mayor and Lopez, 2010).

Puberty

Puberty and sexual maturity of the female occurs at between 12 and 24 months (Wildt *et al.*, 1998), although Henderson (2010) indicates it is reached at 36 months of age. These differences may be due to factors such as daylight hours (latitude), longitude and even prey availability.

Estrous cycle

There is only limited information concerning the estrous cycle of wild felids and especially of the jaguar. One of the most studied females of the Felidae family is the domestic cat, which is sometimes used as a reference. However, it should be considered that this animal is in a different environment than wild felines, in addition to the physiological characteristics of each species, and even among individuals of the same species (Brown *et al.*, 2006).

In jaguars, studies have been conducted to evaluate the hormonal profiles (estradiol and progesterone) of estrous cycles and puberty by collecting fecal samples and found that the cycle length was 38.28 ± 2.52 days, ranging from 25 to 44 days, while sexual maturity was reached within 22 months (Wildt *et al.*, 1998; Viau *et al.*, 2020), the estrous cycle is divided

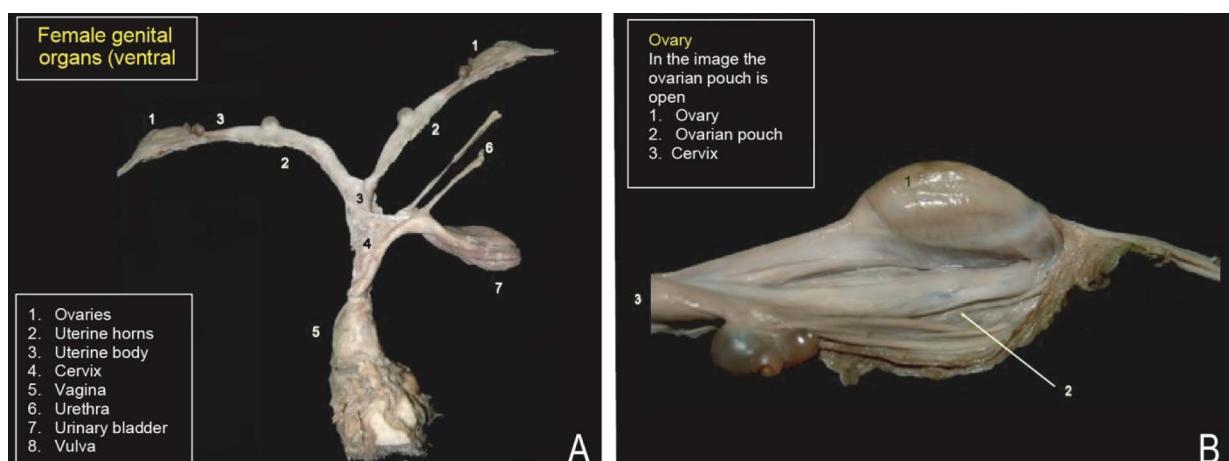


Figure 2. A: Reproductive apparatus of the female jaguar. B: Structure of the ovary of the female jaguar. With permission from Mayor and López (2010). Atlas of Anatomy of wild species of the Peruvian Amazon.

into five stages called proestrus, estrus, diestrus, interestrus and anestrus, with characteristic events as listed below. Proestrus: vocalizations, friction of the female on inanimate objects to attract males without accepting mating. Estrus: mating is accepted. The observable signs are vocalizations in the form of grunts, calling the male, rubbing against inanimate objects, lordosis, rolling on the ground, the female allows the male to sniff the vulva and accommodates it in order to perform the mating (Yescas and Ramírez, 2013). It has been reported that the mean estrous duration of 194 hormonally measured estrous periods in seven females was 6.5 ± 0.3 d (Barnes *et al.*, 2016). Oestrus: in this phase, fertilization is likely to occur, corpora lutea will develop and there will be progesterone secretion to maintain gestation. However, there are females that ovulate, but the oocytes are not fertilized, and present pseudocyesis, which triggers a behavior like that of the pregnant female, caused by the production of progesterone. Interestrus: also known as sexual rest period, in this stage the female does not attract males and it is very common that the signs of estrus disappear. After this period the female resumes sexual activity or goes into anestrus if the reproductive season ends. Anestrus: this occurs during the non-breeding season and is characterized by the absence of ovarian activity and male acceptance (Stornelli, 2007; Barrueta, 2012).

Currently, studies have been conducted to evaluate the hormonal profiles (estradiol and progesterone) of estrous cycles and puberty, by collecting fecal samples and report that the cycle duration was of 38.28 ± 2.52 days, with a range of 25 to 44 days, while sexual maturity was reached at 22 months (Viau *et al.*, 2020).

Reproductive behavior

The behavioral patterns of jaguars in captivity can be grouped into four categories 1) sexual, 2) affiliative, 3) agonistic and 4) individual (Yescas and Ramírez, 2013). Among the sexual ones: vocalization, mating attempts, naso-genital contact, naso-anal contact, genital preening and urine sniffing are the most characteristic activities, it is believed that the reproductive behavior of females in the wild is similar.

Naso-genital contact. The male approaches the female, performing an exploration and sniffs the vulva. Naso-anal contact. It consists of putting the nose of one of them in contact with the anus of the other, although according to Yescas and Ramírez (2013) it is difficult to identify whether the male sniffs the vulva or the anus.

Urine and excreta sniffing. In wild species, it is common for biological fluids (urine, excreta, sweat or saliva) to contain pheromones that induce ethological and/or endocrine responses in animals of the same species (Figure 3).

In reproductive aspects, males can identify if the female is in estrus, depending on the pheromones present in the urine and feces. Pheromone production in the female is cyclical and related to estrogen production (Rigau *et al.*, 2008). Vocalization. It is characterized by loud “grunting” sounds to call the males, who respond in the same way; between grunts and other vocalizations, copulation takes place.

Attempted mating. When the female is receptive, she rubs herself against objects, rolls on the ground and allows mating attempts. If the female is not receptive, she rejects the male in an aggressive manner or otherwise does not accommodate herself adequately for penetration. Full mating.

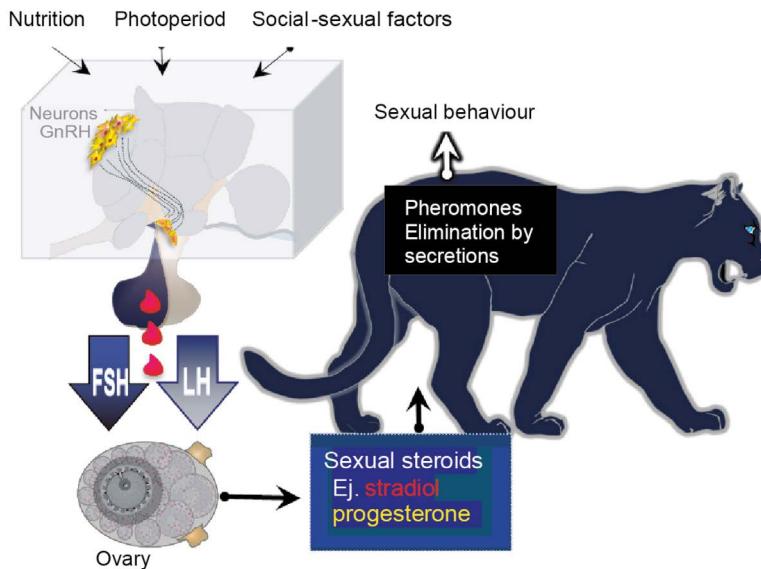


Figure 3. Environmental factors influence on jaguar reproductive behavior. Steroids stimulate the production of pheromones and in the case of the female, secretion is cyclical (Rigau *et al.*, 2008).

The female lies down, the male arrives and presses with his head or forelimbs on the female's abdomen (it is believed that this is to accommodate her for copulation), begins to move his tail, then bites the nape of the female's neck and penetrates her for a period of 7 to 22 seconds and ejaculates. They can copulate several times in a day (Yescas and Ramírez, 2013).

Reproductive process

Reproductive particularities

Ovarian activity in females of the Felidae family is variable, although their reproduction is highly dependent on the photoperiod. They are seasonal polyestrous with induced ovulation. However, some individuals of the domestic cats (*Felis catus*), nebulous panther (*Neofelis nebulosa*), lioness (*Panthera leo*), leopard (*Panthera pardus*) and tigrina (*Leopardus tigrinus*) have been reported to present cases of spontaneous ovulation, a phenomenon that varies among species and individuals of the same species (Brown, 2011). Another characteristic reported in females of this family is the inhibition of ovarian activity when integrated into groups in captivity; it is reported that the socio-sexual environment is important for estrus onset, as is the case of the cheetah (*Acinonyx jubatus*; Brown *et al.*, 1996). In free-living female jaguars, little is known regarding their reproductive behavior; most information is derived from animals in captivity (Hoogesteijn and Mondolfi, 1993), such as lioness (*Panthera leo*; Schaller, 1972) and leopards (*Panthera pardus*; Bailey, 1993). Leuchtenberger *et al.* (2009) pointed out that female jaguars are polyestrous; however, mating is not limited to a single season. This depends on several factors including geographic space, photoperiod, temperature, food availability and social-sexual environment (Feldman and Nelson, 1996). In regard to the photoperiod, Seymour (1989) indicated that in South America, births occur in June, August, November and December, in an average of 100 days of gestation.

It is assumed that mating occurs from April to October. On the other hand, in Mexico, the most recent information of the reproductive season of free-living jaguars mentions that mating occurs in December and January, with births occurring between February and April (Aranda, 1990). However, Leopold (1965) reported that jaguars give birth from July to September and mate from April to July.

Considering geographic location, jaguar mating in Mexico (northern hemisphere) occurs mainly in winter (short days) and in South America (southern hemisphere) in autumn and spring. Therefore, it is believed that the jaguar's reproductive season is determined by day length, so that it is inhibited when day length is longer (more daylight hours) and females have seasonal anestrus and estrus in the short-day season (fewer daylight hours). It seems that as in other mammals, circadian rhythms regulate the neuroendocrine and physiological changes presented by felines in extreme climates, such as hormonal cycles, metabolic rhythms and annual reproduction when they inhabit places with marked seasonality. So, melatonin would also have an important effect on ovarian activity (Wood *et al.*, 2015), whose secretion increases in darkness hours, coincides with the estrus onset (Yamauchi and Matsuura, 2009) and the onset of sexual activity. In tropical areas where there is not such marked seasonality, it seems that reproduction occurs at any time, since light and humidity remain constant. Another factor that may influence the onset of the reproductive season is the food and/or prey availability in extreme climates, where the seasons are well differentiated.

Fertilization, gestation and birth

Although the physiological changes during estrus, fertilization, gestation and birth are already known in domestic animals, information for felines is scarce and contradictory, especially in the case of the jaguar. After insemination, the fertilized oocytes become implanted in the uterine horns and gestation is established with a duration of 91 to 111 days. Female jaguars give birth to up to four cubs, although births of one to two cubs are more common. The cubs are born weighing between 600 and 900 g. The average interval between births is of two years (Ceballos and Oliva, 2005; Ceballos, 2010).

Lactation

The survival of the offspring depends on the mother's ability to produce milk and to obtain prey during rearing. Most female cats have four or five pairs of teats (Barrueta, 2012), although the female margay has only two (Henderson, 2010). From the sixth week, jaguar cubs follow their mothers to learn the secrets of hunting and are weaned at approximately three months, beginning to eat meat, although the lactation period can last from five to six months. During this period, estrous cycles are inhibited (postpartum anestrus). The calves remain with their mother for about 18 months, after which they separate in search of their own territory (Ceballos, 2010).

Hormonal studies

There are large "gaps" in the understanding of reproductive physiology of the jaguar, so it is necessary to generate information and improve the knowledge of the species

to ensure its reproductive success. The analysis of biological samples to characterize over time their hormonal profiles [levels of cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol and prolactin], would allow a better understanding of the annual reproductive activity (reproduction vs. anestrus) of the jaguar (Barrueta, 2012). There are large differences in the levels of reproductive hormones among felids. Thus, for example, it is known that the concentration of progesterone in puma is higher than in domestic cat (Genaro *et al.*, 2007), estradiol is higher in female ocelot than in domestic cat; while, in jaguar, margay, oncilla, Geoffroy's cat, puma and jaguarundi, they are similar and cortisol levels are higher in pumas than in jaguarundis (Genaro *et al.*, 2007).

Assisted reproduction techniques

Oestrus induction and ovulation. In wild females, estrus induction is an alternative that could help to increase the chances of gestation, even females could be artificially inseminated with fresh or frozen semen (Micheletti *et al.*, 2015). This strategy requires the exogenous administration of hormones such as gonadotropin-releasing hormone (GnRh), LH, FSH or gonadotropins, alone or in combination, which modify the endocrine environment and induce ovulation. Although these hormones have been used in free-living animals, the differences that exist between species in relation to the reproductive cycle should be taken into account, for example, spontaneous ovulation in the case of margay and induced ovulation in the case of the female ocelot and tigrina, as in these cases (spontaneous ovulation and induced ovulation), prostaglandins are not an option for wild animals in captivity or in the wild. This is because the action of prostaglandins is limited until day 40 post-ovulation, and they cannot lyse the corpus luteum during the diestrous (Pelican *et al.*, 2006). In pumas and jaguars, porcine FSH has been used to induce follicular development and progestogens to inhibit follicular activity in tigrina (Morato *et al.*, 2000; Pelican *et al.*, 2006).

Oocyte retrieval for in vitro culture. It is a technique that represents the possibility of preserving frozen female gametes as well as pieces of ovarian tissue, follicles, or mature or immature oocytes (Pukazhenthil *et al.*, 2006, Lermen *et al.*, 2009). This technique has allowed to recover ovarian tissue from tigrina, puma and jaguars post mortem (Wiedemann *et al.*, 2013; Baldassare *et al.*, 2015), in the last two species, also, antral follicles (40-90 μ m) were isolated. Laparoscopic aspiration has been used to recover oocytes from jaguar (Morato *et al.*, 2000), tigrina, ocelot (Swanson, 2006) and puma (Baldassare *et al.*, 2015).

In vitro embryo production. This technique consists of oocyte extraction, *in vivo* or *in vitro* maturation, oocyte selection, *in vitro* fertilization and embryo development with the aim of obtaining healthy offspring. An important characteristic to consider is the adjustment of the pharmacological protocols to be used for each species. For example, in the case of wild felines, *in vitro* fertilization has been successfully performed in jaguar, ocelot and tigrina (Swanson and Brown, 2004). In jaguar females, FSH/LH was used and an average of 25 follicles/female were obtained, with more than 80% of good quality oocytes; however, the fertilization rate was less than 25% (Morato *et al.*

al., 2000). In contrast, in ocelot and tigrina treated with eCG/hCG gonadotropins, an average of 10 follicles and between seven and nine good quality oocytes/female were obtained (Swanson *et al.*, 2002).

Embryo cryopreservation. In recent years, great advances have been made in embryo cryopreservation, using slow freezing or conventional vitrification. Protocols developed for domestic species appear to be suitable for embryo cryopreservation in wild felids; ocelot offspring have been obtained using this technique (Swanson and Brown, 2004).

Generation, establishment and preservation of primary somatic cell cultures and cloning. These procedures are routinely used in domestic animals; however, there is little or no use in free-living animals, especially in felines. In addition, cloning for animal conservation is often questioned because of the risk of reducing genetic variability. However, cloning allows to know the principles of nuclear reprogramming, the conservation of cells and tissues for different purposes (reproductive, therapeutic, etc.), clones can also be used in experimental studies where it is necessary to avoid genetic variation.

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CONCLUSIONS

There are no reports of oocyte and embryo cryopreservation in female *Panthera onca*. Assisted reproductive techniques should focus on ovulation induction, control protocols and gamete cryopreservation.

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