UNIVERSIDADE FEDERAL DO ABC

Gislaine Cristina Ceregatti Bonilha Pinto

FISIOLOGIA ESPERMÁTICA DE FELÍDEOS NEOTROPICAIS: REVISÃO BIBLIOGRÁFICA

SANTO ANDRÉ

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Trabalho de Conclusão de Curso apresentado ao Bacharelado em Ciências Biológicas da Universidade Federal do ABC, como requisito para a obtenção do título de Bacharel em Ciências Biológicas.

Orientador: Prof. Dr. Weber Beringui Feitosa

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2020

Sistema de Bibliotecas da Universidade Federal do ABC
Elaborada pelo Sistema de Geração de Ficha Catalográfica da UFABC
com os dados fornecidos pelo(a) autor(a).

Ceregatti Bonilha Pinto, Gislaine Cristina
Fisiologia espermática de felídeos neotropicais : revisão bibliográfica / Gislaine Cristina Ceregatti Bonilha Pinto. — 2020.

55 fls. : il.

Orientador: Weber Beringui Feitosa

Trabalho de Conclusão de Curso — Universidade Federal do ABC, Bacharelado em Ciências Biológicas, Santo André, 2020.

1. Felidae. 2. sêmen. 3. reprodução assistida. 4. neotropicais. I. Feitosa, Weber Beringui. II. Bacharelado em Ciências Biológicas, 2020. III. Título.

ACKNOWLEDGEMENTS

First of all, to my husband, Rafael Pascoal Rodrigues, whose help, comprehension and support were fundamental in my journey in this course. Without him the path to making the dream of my life come true would certainly have been more strenuous.

Second, to my grandmother, Luiza Galerani Stangari (*in memoriam*) who raised me like a mother and provided me to become the woman that I am today.

Third, to each one of my professors at Universidade Federal do ABC, who contributed with their piece of knowledge to build my own, therefore contributing to making me the scientist that I might be someday.

Special thanks to my advisor, Prof. Dr. Weber Beringui Feitosa, for his assistance, patience, guidance and availability during the development of this work and also in laboratory activities. It was a short period but certainly worth and I'm deeply grateful. Also to Profª Drª Marcella Pecora Milazzotto for allowing me in her facilities and Profª Marcela Sorelli Carneiro Ramos for all her support in the Trabalho de Conclusão de Curso.

I'd also like to thank all my students and my coordinators in CNA Santo André, who were always so understanding of my academic duties and all the times I had to reschedule classes and activities in order to coordinate my professional and academic lives.

My thanks also to my friends Samanta Camargo, Carla Magami, Elisa Criado Souza for their support through all these years. I cannot forget to mention too Laurent Rezende, Leonardo Teodoro Jr., Bruno Gumieri, Adrian Ribeiro, Gustavo Duarte, Marcos Silva, Vitor Nascimento, Joice Rodrigues and many others who shared this path with me.

A wholehearted thanks also to Edgard B. Damiani for intellectual and spiritual guidance for more than a decade now, as well as to Fernando C. Lara, for the most incredible experience of my life in the Brazilian Pantanal, which changed my life forever.

My special regards to Lindsey Vansandt for sharing her knowledge as well as her friendship, Profª Drª Cristiane S. Pizzutto for participating in my examination board and Draª Cristina Adania, Jessica Paulino and everybody in Associação Mata Ciliar.

Finally but not less important, to my therapist Marcia Maria Guazzelli, without her support I might have never found the courage to pursue the dream of my life.

And above all of them my infinite gratitude to the Great Mother Goddess. My eternal devotion to Her. You are all that I am, the womb where all creatures come from and where they all shall return to one day. Gratidão.

“Toda a forma de vida é uma manifestação de Deus e está sob os nossos cuidados. Proteja o que é seu - sua fauna sua flora. As plantas e os animais embelezam a terra. São úteis ao homem e representam a riqueza da Pátria. Nunca se deve mutilar, destruir ou deixar que destruam estes bens. Vamos amar nossos animais domésticos. Vamos dar aos selvagens a paz que eles têm direito. Permitamos que enfeitem nossas florestas. Vamos amar os pássaros puros e belos, cantando nas ramagens, voando alegres no espaço ilimitado, como verdadeiros símbolos de liberdade!”

- São Francisco de Assis

‘All things of creation are children of the Father and thus brothers of man. God wants us to help animals, if they need help. Every creature in distress has the same right to be protected.’

- St. Francis of Assisi

RESUMO

Os felinos neotropicais compreendem 10 espécies da família *Felidae*: onça-pintada (*Panthera Onca*), onça-parda (*Puma concolor*), jaguatirica (*Leopardus pardalis*), gato-maracajá (*L. wiedii*), gato-do-mato-pequeno (*L. tigrinus / guttulus*), gato-do-mato-grande (*L. geoffroyi*), gato-palheiro (*L. colocolo*), gato chileno (*L. guigna*), gato andino (*L. jacobita*) e gato-mourisco (*P. yagouaroundi*). A maioria deles é considerada ameaçada de extinção, de acordo com a União Internacional para a Conservação da Natureza (*International Union for Conservation of Nature - IUCN*). Assim, diversas estratégias têm sido empregadas na preservação desses animais, garantindo a continuidade de suas populações, dentre elas está o uso de tecnologias de reprodução assistida (TRA), as quais visam a aumentar a eficiência reprodutiva assim como a troca de material genético entre populações selvagens e cativas. Com isso, técnicas que permitem a coleta, manipulação e preservação de gametas têm sido estudadas. O gameta masculino, o espermatozoide, é de especial interesse por ser produzido em grande quantidade, ser obtido mais facilmente e também por tolerar melhor a criopreservação, permitindo maior disseminação do material genético. Contudo, para garantir sua correta manipulação e uso, é fundamental o conhecimento acerca da espermatogênese e da fisiologia espermática. Contudo, informações sobre a reprodução dos felinos neotropicais é geralmente escassa e dispersa. Assim, esta revisão tem como objetivo fazer uma análise comparativa da fisiologia espermática desses animais, compilando dados das 10 espécies do grupo e comparando-as ao gato doméstico (*Felis catus*) ou outros felídeos. Espera-se que a informação coletada possa contribuir para a reprodução assistida de felinos selvagens e consequentemente para sua conservação.

Palavras-chave: *felidae*, felinos selvagens, reprodução assistida, espermatogênese.

ABSTRACT

The neotropical felids include 10 species of the *Felidae* family which occur in Latin America: the jaguar (*Panthera onca*), the cougar (*Puma concolor*), the ocelot (*Leopardus pardalis*), the margay (*L. wiedii*), the oncilla (*L. tigrinus / guttulus*), the geoffroy cat (*L. geoffroyi*), the pampa's cat (*L. colocolo*), the kodkod (*L. guigna*), the Andean cat (*L. jacobita*) and the jaguarundi (*P. yagouaroundi*). Most of them are considered endangered by the International Union for Conservation of Nature (IUCN). Thus, several strategies have been taken in order to protect these animals and the continuity of their populations, as for example assisted reproductive technologies (ART), which focus on breeding these species in captivity and also promoting exchange of genetic material between captive and wild populations. Therefore, techniques that allow the collection, manipulation, and preservation of gametes have been studied. The male gamete, the sperm, is of special interest as it is produced in larger quantities, is more easily obtainable and also more tolerant to cryopreservation, allowing for increased genetic material dissemination. However, to enable its adequate handling and use, knowledge of spermatogenesis and sperm physiology is fundamental but information on neotropical felids reproduction is usually scarce and dispersed. Therefore, this study focuses on producing a comparative review on the sperm physiology of these animals, contrasting data among the 10 species and comparing it to the domestic cat (*Felis catus*) or other close relative. We hope the information collected here may aid in wild felids assisted reproduction research and consequently in their conservation.

Keywords: cat, *felidae*, spermatogenesis, assisted reproduction.

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1 INTRODUCTION

1.1 Neotropical felid species

The *felidae* family includes 41 species according to the International Union for Conservation of Nature (IUCN) Cat Specialist Group, under the Carnivora class. Of these, 12 wild species occur in the Americas: bobcat (*Lynx rufus*), Canada lynx (*Lynx canadensis*), jaguar (*Panthera onca*), cougar (*Puma concolor*), ocelot (*Leopardus pardalis*), margay (*Leopardus wiedii*), oncilla/tigrina (*Leopardus tigrinus / guttulus*), geoffroy cat (*Leopardus geoffroyi*), pampa's cat (*Leopardus colocolo*), kodkod (*Leopardus guigna*), andean cat (*Leopardus jacobita*), jaguarundi (*Puma yagouaroundi*) (IUCN, 2020). There's scientific divergence whether the tigrina/oncilla, also mentioned as southern and northern tiger cat, comprise a single or two separate species. As past years research has been done ignoring such genetic divergence, in the present review they will be considered to be a single one.

These wild felids occur all along the American territory, with the bobcat and Canada lynx being restricted to the Northern Hemisphere. Therefore, the neotropical felids include 10 species of wild cats, distributed mainly along Central and South America, where 8 of them (except for the kodkod and the Andean cat) are found in Brazil, as shown in **Figure 1**.

Being part of a diversity of cultures in the region, the animals have received a myriad of popular names (as many as 350 for the jaguar, for example), usually originated from pre-Colombian native languages. The scientific names, along some of their Portuguese, Spanish and English popular names are presented in **Table 1**.

1.2 Conservation status

Of the 10 neotropical felids only 4 (cougar, jaguarundi, ocelot and geoffroy cat) figure as "least concern" conservation status according to IUCN, and from these only the latter shows stable populations. All the other ones are classified as near threatened (jaguar, margay, pampas cat), vulnerable (northern tiger cat / southern tiger cat, guigna) or endangered (Andean cat) with decreasing numbers in the wild.

Figure 1 - Spatial distribution of the 10 neotropical felids

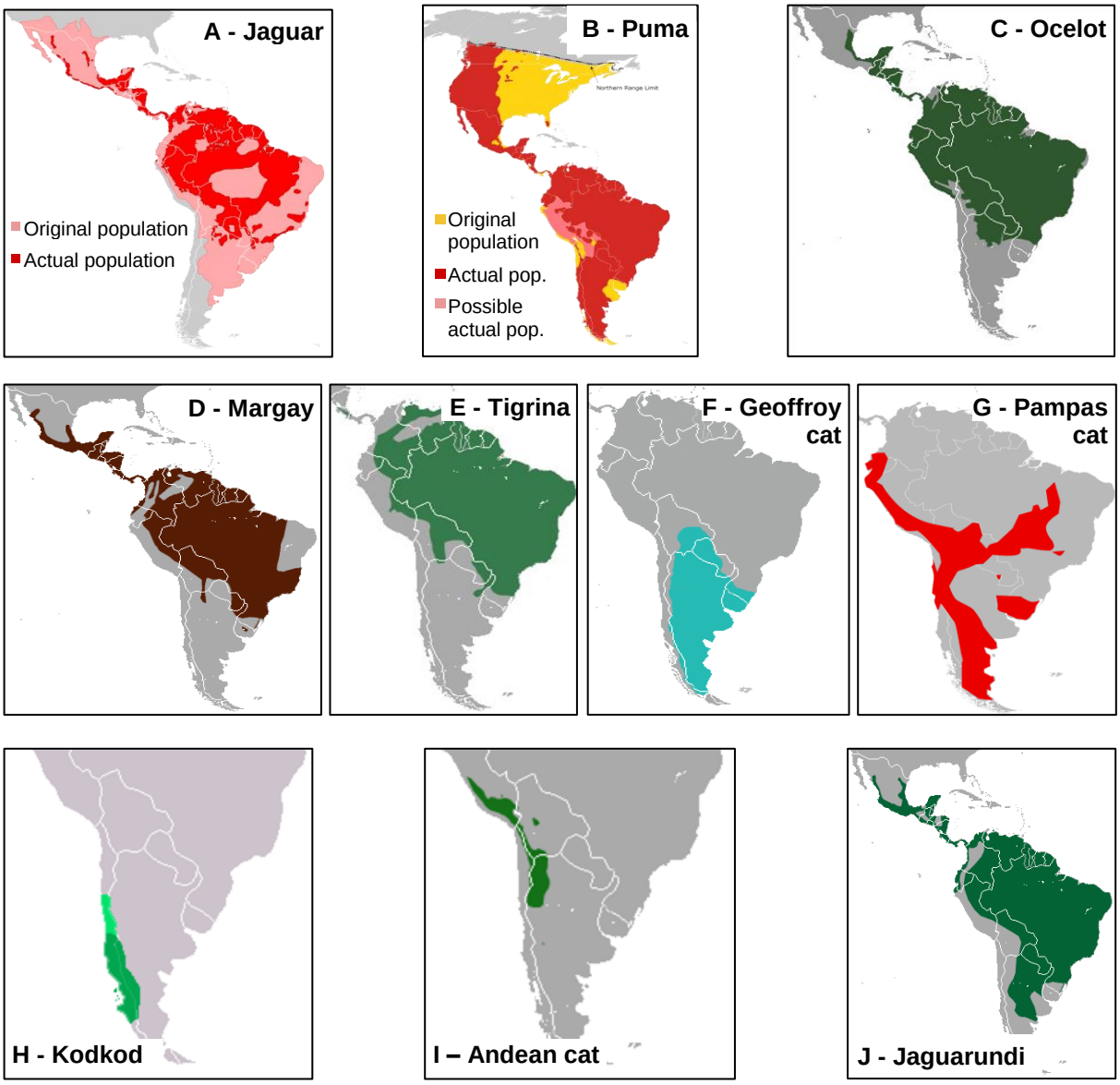

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Table 1 - Scientific and common names in local languages of the 10 neotropical felids

Scientific name	Common name		
	Portuguese	*Spanish*	*English*
Panthera onca	onça, onça-pintada, jaguaretê, iaguaretê, jaguar, pantera	jaguar, yaguar, yaguareté	jaguar
Puma concolor	onça parda, suçuarana / sussuarana, leão baio	puma, león de montaña, león americano	cougar, puma, mountain lion
Leopardus pardalis	jaguatirica	ocelote	ocelot
Leopardus wiedii	gato-maracajá	caucel, gato tigre, tigrillo, maracayá, margay	margay
Leopardus tigrinus / guttulus	gato-do-mato-pequeno	leopardo tigre	oncilla, tigrina, southern tiger cat, northern tiger cat
Leopardus geoffroyi	gato-do-mato-grande	gato montés sudamericano, gato de Geoffroy	geoffroy cat
Leopardus colocolo	gato-palheiro, gato dos pampas, gato-do-pantanal	colocolo, gato de los pajonales, gato de las pampas	pampas cat
Leopardus guigna	gato chileno	huiña, güiña, gato colorado	kodkod
Leopardus jacobita	gato andino	gato andino, titi, chinchay, osjo	Andean cat
Puma yagouaroundi	gato-mourisco, jaguarundi	yaguarundí, gato moro, jaguarundi, jaju, onza, leoncillo, león breñero	jaguarundi

Apex carnivores, such as jaguars, lions (*Panthera leo*), tigers (*P. tigris*), cheetahs (*Acinonyx jubatus*) and other felidae members have been widely used as flagship or umbrella species in conservational efforts. Portraying large, charismatic animals is usually more effective in raising people's awareness towards environmental conservation than showing smaller ones. And as big carnivores usually require just as large extensions of land and sufficient prey populations, the conservational efforts benefit the ecosystem as a whole. Thus, protecting fascinating, attractive flagship species might bring better results when focusing on wildlife protection.

On the other hand there's some discussion on how effective using only these attractive animals could be when developing environment protection initiatives. One of the arguments is that there's bias towards larger animals when distributing funds, in detriment of smaller species (Sergio, 2008). Consequently, little financial support would be applied in the protection of unremarkable, not so charismatic wildlife, while larger amounts of funding are directed to flagship ones. A contrasting point of view is that governmental action is often focused on economically interesting activities, such as ecotourism and trophy hunting, therefore justifying the preference in investing in the conservation of more profitable species.

It is undeniable though that wildlife, whether large or small, should be protected. First because of their universal birthright to life. Secondly because, as already mentioned before, their conservation can help preserve the whole ecosystem where they live, including smaller species, prey communities, the local flora, aquiferous systems and more. Also, animals usually provide ecosystemic services like control of prey populations, seed dispersing, pollination, and serve as quality indicators of the area they inhabit. Finally, they can also be employed in profitable activities such as ecotourism and adventure travel as widely observed in Africa and more recently in Brazil (Tortato, 2017).

1.3 Assisted reproduction

One of the strategies employed for endangered species protection is the use of assisted reproduction technology (ART) to enhance reproductive success as well as allowing for the use of germplasm (sperm and oocytes) in order to increase genetic material exchange. The techniques range from enhancing natural

reproduction by the manipulation of hormonal cycles to artificial insemination (AI), in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT).

In assisted reproduction of wild species, special attention has been given to the male as it presents some advantages in gamete handling when compared to the female. First, its reproductive cell, the spermatozoon, is produced in a much larger quantity than the female oocytes in a given period — providing it with a much higher dissemination capacity — second, it is more easily obtainable, not requiring surgical procedures, and finally it's usually more tolerant to storing and cryopreservation (Silva, 2019).

In conclusion, due to the danger neotropical felids face in the wild and the urge to protect and grant the continuation of their species, the present work has been proposed, in an attempt to provide a comprehensive review on male reproductive physiology and contribute to their conservation through assisted reproduction.

2 OBJECTIVES

The general and specific objectives of this study are listed below.

2.1 Specific objectives

This study aims at performing an extensive comparative review of neotropical felid species sperm research and at moments contrast them with related species such as the domestic cat (*Felis catus*) or another close relative.

2.2 Specific objectives

1. Review felid reproductive system characteristics;
2. Compile available literature on neotropical felid species sperm and assisted reproduction;
3. Perform critical analysis of the collected data;
4. Provide a condensed and comprehensive comparison on seminal characteristics of neotropical felid species.

3 METHODOLOGY

This literature review has been conducted according to the following steps: first, the study focus was defined, which is the spermatic physiology of wild cat species occurring in Latin America. Then, literature was reviewed for articles addressing the theme and desired data was collected. Finally it was grouped under the themes of interest and a critical analysis was performed.

3.1 Article search and selection

Primary searches were conducted using Pubmed database. Whenever little or no data could be found on a given species, supplementary search was performed using Google Scholar.

The searches included the following (but not exclusively) terms: <species scientific name>, <species common name in English, Portuguese or Spanish>, reproduction, reprodução, reproducción, sperm, sêmen, semen, wild cat, wild felid, spermatogenesis, espermatogênese, hormonal, cryopreservation, criopreservação, criopreservación. Searches using Spanish terms were employed mainly to find articles on *L. guigna* and *L. jacobita*, as it is the official language of the countries where these species occur (Argentina, Bolivia, Chile and Peru), though no information on the reproductive physiology of these species could be found.

Articles which contained data related to sperm characteristics were selected for appreciation. Articles that cited values from other publications were discarded and the source publication was used instead.

4 RESULTS AND DISCUSSION

The largest amount of data was found on jaguars, cougars and ocelots while margay, tigrina, geoffroy cat, pampas cat and jaguarundi studies were few. Almost no information could be found on the guigna and the Andean cat, except for conservational researches. The reproductive physiology of these species is almost — if not completely — unknown as they habit less accessible territories such as the Chilean coast and the Andes mountains.

A high number of studies were conducted by Brazilian scientists or in Brazilian institutions like zoos and wildlife protection facilities, either public or private. Articles were published mainly in English language, with a few being written in Portuguese and only one in Spanish. The scientific and English common names were the most used keywords, followed by the Brazilian ones.

Regarding assisted reproduction, the domestic cat is often used as a model mainly where no specific data is available on the species of interest.

4.1 Anatomy

The anatomy of wild felid species reproductive trait is quite similar to the domestic cat (Florindo, 2011) as described for the jaguarundi by Rocha (2017), the ocelot by Carneiro (2010) and the margay's abdominal organs by Grandez (2019). It is composed of testes, epididymides, deferent ducts (vasa deferentia), prostate gland, bulbourethral gland, urethra/urogenital canal and penis.

The testes are two ovoid shaped organs, ventrally positioned to the anus, and are contained in a sac, the scrotum, which is formed by conjunctive tissue. Surrounding the organs a blood vessel complex — the pampiniform plexus— and the cremasteric muscle can be found, forming the spermatic cord, which aids in the thermic regulation through blood flux and adjusting the scrotum distance to the body respectively (Sebastiani, 2005).

Superpositioned to the testis a complex of convoluted tubules are found: the epididymis. These represent a continuation of the testicular rete testis and is where spermatozoa are stored and mature to acquire motility, therefore becoming able to fertilize the oocyte.

The vas deferens connects the epididymis to the urethra inside the prostate,

where the canals fuse to become the urogenital canal. Then it extends through the penis and is where sperm is secreted from and urine is excreted from the bladder. Along with the prostate, another accessory gland, the bulbourethral gland, produces the seminal fluid, a solution rich in minerals and proteins which is added to epididymal spermatozoa in the ejaculation event to form the semen or ejaculate. Interestingly, in contrast to other mammals like the man, felids don't show a seminal vesicle.

The feline penis also shows some particularities. It is small when compared to other taxa such as canids, for example the domestic dog (*Canis familiaris*). It is also more hidden, being contained in the urogenital aperture just inferior to the scrotum, and is exposed only during mating. Finally, the organ is covered with keratinized spines, which are believed to have the function of inducing ovulation in the female (coitus stimulation). An exception to this characteristic is the margay (L. wiedii), where males don't present penile spines and females present spontaneous ovulation (Valverde, 2019; Swanson, 2004). Jaguars also seem to present smaller spines, and spontaneus ovulation has been recorded in this species when females are housed with sensorial stimulation by nearby males (Jorge-Neto, 2020).

4.2 The testis

In amniotes, the testes is where sperm production takes place. Internally, the organs are formed by a series of convoluted tubules — the seminiferous tubules — and Sertoli and Leydig cells, the last being distributed in the interstitial space. The tubules are lined with primordial germ cells, the spermatogonia, which undergo a series of cellular events to form a functional spermatozoon, in a process named spermatogenesis (Clermont, 1976).

Being the central unit where spermatozoa are produced, the testes are usually inspected for size and mass and other characteristics when conducting assisted reproduction research.

4.2.1 Testis volume

Testis volume is one of the organ's characteristics which is evaluated when considering an individual for its employment in assisted reproduction — or even in

routine biometry. The consensus is that larger organs usually reflect in higher sperm production therefore better reproductive capacities. (Noorafshan, 2014; Azevedo, 2008; Swanson 2003). To estimate their volume, the most commonly used formula is that of the prolate spheroid:

$$V = 4/3\ \pi ab^2, \text{ where a=length/2 and b=width/2,}$$

(Bailey, 1998; Wildt, 1982)

In domestic cats the mean testicular volume has been documented as ranging from V=0.55cm³ in individuals less than 6 months old (pre-puberty) to V=2.66cm³ in individuals more than 12 months old (adults) (Leme, 2018). Hence, a 5 times enlargement in testes volume can be observed when comparing prepubescent and pubescent individuals, evidentiating the increase in testicular activity during the transition from one life phase to the next.

The data found on neotropical cats testicular volumes are depicted in **Table 2**, with results grouped by species and listed from the most recent study one to the oldest.

From the numbers we notice that in wild species testes volumes may vary from as big as 62.43cm³ in the jaguar (Valverde, 2019) to as small as 1.53 ± 0.39cm³ in the tigrina (Balarini, 2012) naturally accompanying the difference in the animals' body sizes.

When comparing values for each species, a large variation can be seen in published data for the ocelot (17.1±1.6cm³ to 59.28 ± 6.90cm³), a variation of 246.67 ± 0.15%, and the tigrina (1.53 ± 0.39cm³ to 4.2±0.2cm³, a 174,51 ± 0,26% variation). Something similar can be seen for the jaguarundi (3.0 ± 0.3cm³ to 7.12cm³, 137.33 ± 0.1% variation). The large differences in collected data evidentiates the need for more research on these animals or perhaps to include testicular volume measurement in routine biometry procedures. Having more available data could improve knowledge on their reproduction and contribute to breeding efforts.

In the jaguar smaller differences were observed (max. 62.43cm³, min. 44.4 ± 2.0cm³; a 40.61 ± 0.05% variation). The reason might be that it is a largely studied species, being often found in captivity as well as being more extensively researched in the wild than its relatives

Table 2 - Total testicular volumes and values variation

Species	Total volume (cm^3)	Variation (%)
Panthera onca	62.43[a], 44.4 ± 2.0[b], 46.2 ± 2.9[c], 51.4 ± 2.4[d]	40.61 ± 0.05
Puma concolor	18.2 ± 0.7[c]	
Leopardus pardalis	17.1 ± 1.6[e], 59.28 ± 6.90[f], 22.0 ± 1.3[c], 32 ± 1.3[g]	246.67± 0.15
Leopardus wiedii	4.37 ± 0.9[h], 5.35[a], 4.8 ± 0.4[c], 6.2 ± 0.2[g]	41.88 ± 0.21
Leopardus tigrinus / guttulus	1.53 ± 0.39[i], 2.3[j], 2.8 ± 0.3[c], 4.2 ± 0.2[g]	174.51 ± 0.26
Leopardus geoffroyi	5.5 ± 0.4[c]	-
Leopardus colocolo	2.8 ± 0.7[c]	-
Leopardus guigna	-	-
Leopardus jacobita	-	-
Puma yagouaroundi	3.0 ± 0.3[c], 7.12[a]	137.33 ± 0.1

Sources: [a](Valverde, 2019), [b](Morato, 2004), [c](Swanson, 2003), [d](Paz, 2000), [e](Sarti, 2009), [f](Stoops, 2007), [g](Morais, 2002), [h](Erdmann, 2020), [i](Balarini, 2012), [j] (Erdmann, 2005).

Similar divergences in values could be observed for the margay (41.88 ± 0.21%) though this species is not as easily found in captivity and little research is done about it in the wild. Therefore the variability may reflect better the natural dissimilarities among individuals.

Only two studies on the jaguarundi could be found, and only one on the

geoffroy cat and the pampas cat, which didn't allow for comparison.

Finally, no data seems to be available on kodkod and Andean cat (Andrews, 2019) as no representatives could be found in captivity (Swanson, 2003) and there seem to be no studies in the wild regarding their reproductive biology.

On the other hand, though a variety of studies report larger testes as an indicator of high fertility, an inverse correlation between the number and quality of produced sperm and organ size has been found in domestic cats (Gutiérrez-Reinoso, 2016; Neubauer, 2004) thus questioning the parameter efficacy on determining an individual's reproductive soundness and requiring that other characteristics be assessed.

4.2.2 Gonadosomatic index

The ratio between the animal's weight and its testicular mass, known as the gonadosomatic index (GSI), has also been used to evaluate reproductive soundness in mammals (Amann, 1970). A larger relation between the body mass and testes size is believed to be related to larger energy expenditure in gamete production, therefore signalling better reproduction success.

Another correlation seems to exist between gonads size and the mating system. As stated by Short (1997), species which show promiscuous (an individual of one sex mates which several individuals of the opposite sex) or polygamous (an individual of one sex has an exclusive relationship with two or more individuals of the opposite sex) often show higher GSIs, first because larger sperm quantities are required for an increased number of mating events and also because sperm competition would occur — as the female tends to mate with multiple partners — and a male who ejaculated a larger volume of sperm would have higher reproductive success. **Table 3** summarizes the collected data for the gonadosomatic index.

In the domestic cat the GSI is 0.08, as described by França (2003). This number stands between the lowest values, as found for the tigrina (0.06) and the highest ones, as for the ocelot (0.16). A large variation can be observed in numbers reported for the jaguar, with values ranging from 0.034 (Azevedo 2006) to 0.11 (Valverde 2019). Such discrepancy is questioned in this review and one hypothesis was that evaluated animals could come from different origins (wild or captive born). While in the first study subjects were from wild origin and zoo kept, in the second

Table 3 - Gonadosomatic index (GSI)

Species	*Gonadosomatic index*
Panthera onca	0.11[a], 0.034[b]
Puma concolor	0.033[c]
Leopardus pardalis	0.16[d], 0.12[e]
Leopardus wiedii	0.14[a]
Leopardus tigrinus / guttulus	0.06[f]
Leopardus geoffroyi	-
Leopardus colocolo	-
Leopardus guigna	-
Leopardus jacobita	-
Puma yagouaroundi	0.12[a]

Sources: [a](Valverde, 2019), [b](Azevedo, 2006), [c](Leite, 2002), [d](Silva, 2010), [e](Sarti, 2009), [f](Balarini, 2012).

study no information about the animals' origin was available, making it impossible to infer such a relationship between the findings. Another questioning was the calculation methods used by both groups. While Azevedo *et al.* used the prolate spheroid formula to estimate the animals testicular volume, Valverde *et al.* employed the sphere formula ($V = 4/3\pi r^3$), which is a less precise method if we consider that the testis length is usually larger than its width. Also, in the first study the albuginea and mediastinum (conjunctive tissues surrounding the testis) were estimated in 18% and discounted from the calculations. Finally, the testicular density values used in each study were different, while D=1g/mL was used by Azevedo *et al.*, a density of D=1.046g/mL was employed by Valverde's group. All these methodological differences, in addition to the lack of standard deviations in Valverde *et al.*'s study, contribute to the disparity in the numbers. A standardized method should be used in order to allow for proper comparison.

Another critic is that such measure is based on a ratio between the animal's total and testicular masses, and as a consequence overweigh individuals would show a lower index, not necessarily indicating reduced testes volume.

For the other species, similar low GSIs were found for the cougar and tigrina, with values of 0.033 (Leite, 2002) and 0.06 (Balarini, 2012) respectively, all of them kept in zoos but without data on their origin.

The margay and jaguarundi showed close numbers, where gonadosomatic indexes reached 0.14 (Valverde 2019) for the first and 0.12 for the last (Valverde 2019), though careful use should be made of such values as the calculation method has already been criticized in this work.

Surprisingly, small cats like the ocelot showed the highest proportions between body mass and testicular weight, differing from animals from wild origin and captive ones, with values ranging from 0.12 (Sarti, 2009) to 0.16 (Silva 2010) respectively.

The findings corroborate the studies of Kenagy (1986) where it was observed that large mammals tend to allocate less energy in testicular tissue in opposition to the expenditure seen in small ones.

Regarding the mating system, studies on felid spatial occupation show a contradictory tendency. Jaguars were found to present a polygynous behavior — one male mates with many females — or a promiscuous one (Cavalcanti, 2012; Gonzalez-Borrajo, 2017) therefore contradicting Short's (1997) hypothesis that non-monogamous animals would have larger testicles. The same happens to other large felids such as the cougar, which shows a polygynous mating behavior (Gonzalez-Borrajo, 2017) and a low GSI (0.033).

In contrast, the ocelot, a small felid, which showed monogamous relationships in one study conducted in Peru (Emmons, 1988) presents a high GSI (0.14 on average) if compared to the jaguar and the cougar. Again, the assumption that monogamous species have smaller testicles holds not to be true, at least for these felids. More extensive studies are required on the species mating habits and their reproductive organs biometry to allow the inference of a relationship between the characteristics.

4.3 The Sperm

The sperm, as the male genetic information carrier, has been widely studied for a large number of species including extensive research in mammals and farm animals, mainly because it is more accessible, more tolerable to preservation and also produced in a large quantity, if compared to the female's gamete. Therefore another focus on the evaluation of individuals reproductive capacities are related to the sperm quality.

4.3.1 Spermatogenesis and the seminiferous epithelium cycle

The spermatozoon derives from fundamental cells, the spermatogonia, which are found in the internal surface of the seminiferous tubules of the testis. The process from which a spermatogonia gives rise to spermatozoa is called spermatogenesis (Clermont, 1976).

In the first step spermatogonia undergo a mitotic division promoting their own renewal and also the formation of a spermatocyte, the first differentiated cell (Dalia, 2019). The next steps include two meiotic divisions to produce a haploid cell, the spermatids, as gametes only carry half of the genetic material of the parents, forming a diploid embryo when the spermatozoon fertilizes the oocyte.

Before a spermatozoon arises, round spermatids still go through cytological and morphological transformations: fundamental structures like the acrosome, the midpiece and the flagellum are formed. The first observable modification is the condensing and migration of the cell nucleus to the periphery of the cell. Later, a modified lysosome, the acrosome, is formed from the Golgi complex and is attached to the nucleus, in superposition to the cell membrane. The flagellum is assembled from the axoneme, a microtubules center derived from the cell centrioles. Later on in the cell development, this structure gives rise to nine dense fibers, which elongate to form the cell's tail, distally to the acrosome. The stretching of the cell cytoplasm forms its neck, or the midpiece, where mitochondria are located in order to provide ATP for flagellar movement. Finally, most of the cell remaining content is shed, in a structure called the cytoplasmic droplet (Kretser, 1998).

Spermatozoa is then released into the seminiferous tubule lumen in the event of spermiation (Dalia, 2019). They are not able to fertilize an oocyte yet and have to undergo maturation in the epididymis.

Early studies of seminiferous tubules epithelium showed that cells going through spermatogenesis were organized in well-defined groups, leading to the concept that the processes occurred in waves, or seminiferous epithelium cycles. Spermatogenesis is a well conserved process in mammals, therefore the domestic cat, so it is expected that similar processes take place in wild felids.

It's been stated that in mammals at least 4 cycles are necessary for the release of a spermatozoon (ROOSEN-RUNGE, 1962), being the average length of each cycle 16 days in man (Heller, 1963), 6.5 days in rats (Leblond, 1952) and 10.4

in the domestic cat (França, 2003). More recent studies have shown that exactly 4.5 cycles occur during spermatogenesis in mammals, allowing the calculation of each process' length given that the duration of the other one is known. Therefore, spermatogenesis in the domestic cat would take 46.8 days (França, 2003).

Also, the cycles' duration seems to be determined by the germ cell genotype, as proven with spermatogonial transplantation from rats to mice, where each cycle took 6.5 days, as expected for donor cells. In short, cycles' and spermatogenesis length are unique for each species (Hess, 2009).

Regarding neotropical felids, little information could be found on the duration of their spermatogenesis and the seminiferous epithelium cycles. The collected values can be found on **Table 4.**

In the largest species of the group, the jaguar, spermatogenesis takes 57.6 ± 0.07 days. Consequently, the seminiferous epithelium cycle could be estimated to take 12.8 ± 0.01 days (Costa, 2008).

The shortest lengths were found in the jaguarundi, requiring 8.4 days for a cycle to complete and 37.8 days for a gamete to emerge (Silva, 2014). And although they may be similar in size to the ocelot, the process is almost 20 days shorter.

Table 4 - Spermatogenesis and Seminiferous epithelium cycle duration

Scientific name	Spermatogenesis duration (days)	Seminiferous Epithelium Cycle duration (days)
Panthera onca	57.6 ± 0.07[a]	12.8 ± 0.01[a]
Puma concolor	44.5[b]	9.89[b]
Leopardus pardalis	56.3 ± 1.9[c]	12.5 ± 0.4[c]
Leopardus wiedii	-	-
Leopardus tigrinus / guttulus	41.37[d]	9.19[d]
Leopardus geoffroyi	-	-
Leopardus colocolo	-	-
Leopardus guigna	-	-
Leopardus jacobita	-	-
Puma yagouaroundi	37.8[e]	8.4[e]

Sources: [a](Costa, 2008), [b](Leite, 2006), [c](Silva, 2010), [d](Balarini, 2012), [e](Silva, 2014)

These particularities in spermatogenesis length, besides evidentiating that the duration of the process is indeed particular to each species, show that differences in the sperm production metabolism might exist and specific collecting schedules should be employed in order to achieve the best results.

4.3.2 Daily production

Another measure that can be used when assessing species reproductive capacities is the daily sperm production. The values are usually calculated using the number of spermatozoa produced in a day per gram of testis, it is, a correlation between an individual's testes mass and the total amount of sperm generated in a day (França, 2003). This correlation is also called the spermatogenic efficiency. **Table 5** summarizes the values found for the daily sperm production of a few neotropical felids.

In the domestic cat, approximately 15.7 ± 1.6x10^6 spermatozoa are produced per gram of testis per day (França, 2003). Similar values have been found for the jaguar, where 17 ± 1.2x10^6 gametes are produced by testis gram daily (Costa, 2008) and the ocelot, with a daily output of 26.8 ± 5.3x10^6 cells (Leite, 2003).

Table 5 - Estimated daily sperm production

Scientific name	Daily production (testis gram/day)
Panthera onca	17 ± 1.2 x$10^{6\ a}$
Puma concolor	26.8 x10^6 ± 5.3 x$10^{6\ b}$
Leopardus pardalis	18.3-1 ± x$10^{6\ c}$
Leopardus wiedii	-
Leopardus tigrinus / guttulus	-
Leopardus geoffroyi	-
Leopardus colocolo	-
Leopardus guigna	-
Leopardus jacobita	-
Puma yagouaroundi	51.52 x$10^{6\ d}$

Sources: [a](Costa, 2008), [b](Leite, 2003), [c](Silva, 2010), [d](Silva, 2014).

Though similarly sized to the jaguar, the cougar showed higher spermatogenic efficiency than the first, yielding 26.8 ± 5.3x10^6 spermatozoa per testis gram per day (Leite, 2003).

The highest efficiency has been seen in the jaguarundi, which produces 51.52x10^6 sperm cells per testis gram daily (Silva, 2014), as also noticed by its short spermatogenesis and seminiferous epithelium cycles.

4.3.3 Sperm evaluation

When processing sperm, some characteristics are evaluated to deem it adequate or not for use in assisted reproduction (Curry, 2007; Pukazhenthi, 2001). Assessed parameters usually include concentration, motility and cell morphology and possible contamination (Kheirkhah, 2017).

Concentration is depicted by uniform and opaque color of the ejaculate (Ax, 2000) — meaning the presence of a large number of spermatozoa, a desirable characteristic when carrying out ART procedures — and confirmed with microscopical inspection.

Sperm motility is also evaluated. In natural intercourse sperm cells swim up the female's reproductive tract in order to reach the oocyte so vigorous, forward motility is required. Although such demand may be reduced in IVF or ICSI procedures, such parameter is valuable when performing intravaginal or transcervical depositions or pairing up individuals in traditional breeding attempts (Lasley 1994). Also, sperm with reduced motility are more prone to death soon after collection or during cryopreservation.

Cell morphology is assessed as well, to verify abnormal or damaged structures such as flagellum, cytoplasmic droplet, acrosome and chromatin, which could impair spermatozoa ability to fertilize the oocyte and prevent embryo development.

Sperm should also be free from contaminants as for example urine, hair or dirt, as these may affect cell quality.

Sperm concentration

Ejaculate concentrations showed the largest variations among the surveyed parameters. The reported values for the species on which spermatic concentrations could be found are shown in **Table 6**.

In the retrieved researches, jaguar sperm numbers could possibly be as low as $3.9x10^6/ml^{-1}$ (4 captive adults; Morato, 1999) to as high as $1280.7x10^6/ml^{-1}$ in 5 wild animals, reaching an impressive amount of $5{,}115x10^6/ml^{-1}$ in one outlier captive individual (de Araújo, 2018). In the same study, two wild subjects showed almost three times as much sperm in ejaculate than the mean captive ones. Here a single urethral catheterization after pharmacological induction with medetomidine was

Table 6 - Sperm concentration

Scientific name	Sperm concentration ($x10^6/ml^{-1}$)
Panthera onca	316.6^{x}, $1280.7^{y,a}$
	13.8 ± 4.2^{b}
	$6.3 \pm 2.4^{x,c}$
	59.3 ± 12.8^{x}, $152.0 \pm 88.0^{y,d}$
	3.9 ± 0.7^{e}
	$6.20 \pm 3.03^{x,f}$
Puma concolor	205 ± 141.77^{g}
	15.6 ± 4.5^{h}
Leopardus pardalis	28.0 ± 17^{i}
	190.2 ± 73.2^{j}
	169.2 ± 54.90^{k}
	53.8 ± 17.9^{h}
Leopardus wiedii	45.1 ± 12.3^{l}
	14.2 ± 5.3^{h}
	75.6 ± 11.0^{m}
Leopardus tigrinus / guttulus	78.5 ± 33.8^{l}
	83.0 ± 35.5^{h}
	242.8 ± 85.2^{j}
Leopardus geoffroyi	66.5 ± 24.4^{h}
Leopardus colocolo	364.0 ± 326.0^{h}
Puma yagouaroundi	7.2 ± 4.0^{h}

Sources: [a](de Araújo, 2018), [b](Paz, 2006), [c](Morato, 2004), [d](Morato, 2001), [e](Morato , 1999), [f](Morato, 1998), [g](Deco-Souza, 2013), [h](Swanson, 2003), [i](Silva, 2019), [j](Baudi, 2008), [k](Stoops, 2007), [l](Erdmann, 2020), [m](Morais, 2002), x = captive individuals, y = wild individuals.

performed, while 6 electroejaculation (EE) procedures (every two months, for one year) were conducted in the first, which may compromise results comparisons

Cougars presented spermatic concentrations ranging from $15.6x10^6/ml^{-1}$ (Swanson, 2003) to maximum of $400x10^6/ml^{-1}$(Deco-Souza, 2013). However the first study was the most extensive one, using 35 individuals from 9 different institutions throughout South America. Both studies conducted a single electroejaculation procedure as a collection method.

Another large variation was seen for the ocelot. In a study conducted by Silva (2019) results yielded a concentration of $28.0 \pm 17x10^6/ml^{-1}$. In another one, performed by Baudi (2008), ejaculates obtained from 3 captive individuals contained $190.2 \pm 73.2x10^6/ml^{-1}$ spermatozoa on average. The numbers yield a sixfold difference between the findings of each research.

Likewise, a significant variability could be seen in the tigrina. The lowest value found for this species was $78.5 \pm 33.8x10^6/ml^{-1}$ (Silva, 2019) (collection method not mentioned) while the highest was $242.8 \pm 85.2x10^6/ml^{-1}$, obtained through 2 electroejaculation procedures at least two months apart (Baudi, 2008).

The margay also showed highly different spermatic concentration values ranging from $14.2 \pm 5.3x10^6/ml^{-1}$ (Swanson, 2003) to $75.6 \pm 11.0x10^6/ml^{-1}$ (Morais, 2002), both by employing electroejaculation as a collection method but with one collection being performed in the first study and 14 monthly procedures being conducted in the last.

For the geoffroy cat, pampas cat and jaguarundi species the only available information is that from Swanson (2003). Another noticeable finding reported in this study is that small sized felids produced higher sperm concentrations, on average, than the larger ones.

Finally, due to the differences in collection methods and their frequency as well as subjects origins, performing a proper, vertical comparative analysis is problematic. Such numbers can be used as a guide on potential sperm concentrations for each species but not as a standard to be followed.

Morphological abnormalities

Morphology of spermatozoa is also evaluated when assessing sperm characteristics. Frequent deformations involve structures like the flagellum, the midpiece and the acrosome. Damages to the chromatin are also observed as well as

retention of the cytoplasmic droplet (Pukazhenthi, 2001).

In the domestic cat, the most common flagellum abnormalities are bent tails (usually close to the mid-piece but can also occur mid-flagellum), coiled tails and double bent tails. The midpiece may show structural abnormalities like mitochondrial sheath aplasia or defective contour of the head. Acrosomes can also suffer malformations (in a doorknob shape, for example) or swelling. Retention of the cytoplasmic droplet, proximal or distal to the sperm head, may also occur (Axner, 2007). Such sperm defects impair cells ability to swim and as a consequence their fertilizing capacity, reducing reproductive success in the wild as well as in captivity.

Current technology provides automated tools for more precise, standardized sperm evaluation using computer assisted sperm morphometry analysis (CASMA) equipment, allowing for more comprehensive assessment of cells integrity and form (Yániz, 2015).

Teratospermia: Sertoli cells and the hormonal regulation of spermatogenesis

Teratospermia is a phenomenon where less than 40% of the spermatozoa of an ejaculate show normal morphology. The phenomenon is particularly common in felids when compared to other mammals (Wildt, 1994), occurring in more than 70% of the taxon species (Pukazhenthi, 2001).

Along with reduced number of cells, research has shown that teratospermic domestic cats' sperm present reduced fertilizing capacity such as low capacity to undergo capacitation, acrosome reaction, penetrate the zona-pellucida and finally survive cryopreservation, therefore compromising its use in assisted reproduction. Chromatin damage has also been observed in such donors, but surprisingly no impairment in fertilization and embryo development was seen after intracytoplasmic injection (Pukazhenthi, 2006).

Though sperm production in the domestic cat has been proved to be increased in teratospermic donors (Jewgenow, 2013; Pukazhenthi, 2006), probably due to larger testicles and higher amount of seminiferous epithelium and Sertoli cells, combined with reduced apoptosis during spermatogenesis. The ejaculates also showed a higher proportion of defective cells, leading to the evidence that an increase in quantity would occur at the expense of sperm quality. Abnormal Sertoli cells number or activity has also been documented (Jewgenow, 2013). Surprisingly,

higher sperm count/ml were found in teratospermic domestic cats accompanied by lower testosterone levels (Howard, 2009).

Inbreeding is believed to be a key factor causing teratospermia in felids (Howard, 2009; Pukazhenthi, 2006). In wild cats, reproduction between closely related individuals mainly due to populations isolation, as seen in the Asian lion (*Panthera leo persica*) in Western India, or reduced number of individuals like the Florida Panther (*Felis concolor coryi*) in Southern Florida, has proved to be highly deleterious. Research has reported that such populations either produce a large number of sterile offspring (African lion) or the remaining individuals present reduced genetic variability (<85% than other cougar populations), deeming them susceptible to even the faintest genetic or habitat perturbations (Wildt, 1994).

Hormonal regulation of spermatogenesis

In males, the reproductive activity is regulated by four hormones: gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone. The hormones are produced and released according to positive and negative feedback systems that contribute to the control of spermatogenesis.

GnRH is produced and secreted by the hypothalamus and exerts its actions on the anterior pituitary. This gland produces and releases LH and FSH, which will act on Leydig cells and Sertoli cells respectively (Chłopik, 2019).

Leydig cells are located in the interstitial space of seminiferous tubules in the testes, being responsible for the production of testosterone, the most important hormone involved in the control of spermatogenesis.

Sertoli cells are located at the internal surface of the tubules, along with spermatogonia, and are sensitive to testosterone. Early research showed that these cells were intimately related to the germ cells and their posterior stages until the release of spermatozoon (Roosen-Runge, 1962; Clermont, 1972). Interestingly enough, later studies discovered that testosterone receptors are present in Leydig, Sertoli and peritubular cells but not in spermatogonia, evidentiating their supportive role in the development of sperm (Walker, 2009).

Sertoli cells and teratospermia

The ratio between round spermatids and Sertoli cells, also known as their efficiency, represents the number of developing spermatozoa that a single Sertoli cell has to support during spermatogenesis. As expected, an increased ratio means that a larger number of spermatids would depend on each single Sertoli cell, therefore reducing their capacity in supporting each spermatozoon. This increased ratio has been related to be one possible cause of teratospermia: the augmented number of spermatids would unquestionably result in a larger number of spermatozoa, but at the expense of its morphological quality due to reduced Sertoli cell supportive capacity.

The relationship has been demonstrated by Jewgenow (2013) for the domestic cat, comparing permanent teratospermic, three-generation inbred individuals, and random teratospermic toms from a local shelter. In the results, permanent teratospermic cats showed a 13.4 ± 0.5 round spermatid/Sertoli cell ratio while normospermic ones presented a 6.1±0.4 ratio.

The study has also discussed the relationship between the high number of morphologically abnormal spermatozoa and lower Sertoli cell efficiency for wild felid species. Teratospermic lions and Siberian tigers (*Panthera tigris altaica*) showed ratios ranging from [7.2 ± 2.7 - 12.5 ± 1.9] and [7.0 ± 2.2 - 9.3 ± 2.2] respectively and one Amur leopard (*Panthera pardus orientalis*) also presented an increased ratio of 10.7±1.4, almost twice as much as seen in the normospermic domestic cat.

As for neotropical felids, normospermic Sertoli efficiency values were only found for the ocelot and the jaguar, being those 4.5 and 11.0, respectively (Silva, 2010; de Azevedo, 2010). More studies are required to establish a standard normospermic ratio and also define it for other felid species though there clearly seems to be a relationship between high round spermatids/Sertoli cell proportion and teratospermia according to the reviewed data.

Hormonal monitoring for assisted reproduction

Due to their role in spermatogenesis and related processes, monitoring of hormonal fluctuations is used to study reproductive activity. Conventional methods usually evaluate plasmatic concentrations of hormones (Müller, 2012), which require sedation and obtaining blood samples from subjects.

A more contemporary approach is the use of less invasive methods such as faecal analysis (Brown, 1996). Besides not demanding physical contention of the animals, no specialized professionals are needed, as samples can be collected by feeders or zookeepers. Also, it avoids the influence variations of stress-related hormones like epinephrine and cortisol, provided that a standardized sample collection protocol be used to prevent natural daily hormonal variation from influencing the evaluations.

4.4 Seasonal variations in sperm production

Spermatogenic activity may also be influenced by annual seasons, as some research has shown, both for domestic cats and wild ones.

In the domestic species, variations seem to be related to summer and winter. According to one study conducted by Blottner (2007) with 129 toms in Germany, the subjects presented increased testosterone concentrations in spring and decreased levels in fall, with a peak of sperm motility being reached in March, which represents the beginning of spring in the Northern Hemisphere. A biological explanation for such variation, based on natural selection, may be that being the males able to fertilize the females in this period, offspring would be born in early May, which is right before summer (with their gestation taking about 60-70 days). That would mean a higher availability of food, as fauna in general tend to be reproducing at such period of the year, being more active and with increased populations, meaning more prey with which the female can feed her cubs.

Photoperiods may as well have a role in augmented sperm production. Other studies on this subject using the domestic species found that epididymal sperm morphology, motility, cell count and testosterone levels are improved in periods when days are longer than nights, this is, in spring and summer (Nuñez-Favre, 2012). Stornelli (2009) also reports a higher number of tailed and mature spermatids during long days periods.

For the neotropical felids such as the jaguar, which inhabit regions where seasons limits are not always clearly perceptible (Southern Hemisphere), no such disparities could be observed when comparing sperm parameters or steroidogenic activity and seasonal variation (Morato, 2004). Interestingly though, there seemed to be some difference when results were grouped in wet (Sep-Feb) and dry periods

(Mar-Aug), with hormonal levels being higher in the first. Similar to what has been hypothesized for the domestic cat, a possible reason for such observations may be related to prey availability where jaguars are found: mainly the Pantanal and the Amazon, territories where rains are more abundant in spring and summer, with increased food availability for herbivores and as a consequence, for carnivores and their offspring.

The same has not been observed for species like the ocelot, margay and tigrina, which follow a pattern more similar to that of the domestic cat with sperm production peaks in summer (Morais, 2001). More studies on these species' reproductive characteristics or their populations dynamics must be done so a relationship between steroidogenic activity and seasonality can be established.

4.5 Cryopreservation

As wild cats' numbers decrease in nature and assisted reproduction is used as an attempt to keep viable populations, such initiatives often face the challenge of maintaining genetic variability. To allow for the introduction of founder genes into captive populations and also the preservation of endangered species cell samples, the creation of genome resource banks has been proposed (Amstislavsky, 2017; Swanson, 2007).

Such banks may be composed of different tissues such as germplasms, somatic cells and embryos, but considerable effort has been put in obtaining, processing and preserving sperm, as it is usually simply obtainable and storable.

In order to maintain sperm characteristics such as cell motility and the integrity of its structures, specific procedures and preserving substances — cryoprotectants — have been employed when submitting cells to cryopreservation.

For that, two different approaches are possible: cooling and freezing. Cooling usually means storing samples in refrigerators for immediate use or short period storage, under temperatures of around 5ºC. Freezing requires lower temperatures for storage, mainly below -195ºC, using liquid nitrogen (LN_2). Regarding the techniques, a few of them have been developed, such as pelleting, where sample drops are directly dispensed on dry ice being posteriorly stored in vials in LN_2; vapor freezing, with semen being deposited in straws and frozen in LN_2 vapor; or dry shipper freezing, where vials are first cooled in empty but fully charged dry shippers before

being plunged into LN_2 (Roth, 1999).

In the domestic cat, semen has been reported to survive cooling for periods ranging from 24hrs up to 14 days, with successful embryo development up to blastocyst (Luvoni, 2003). In wild species such an approach is not very popular as samples are mainly frozen to allow for their posterior use.

Regarding cryoprotectants, these focus mainly at maintaining cell membrane stability and solution's osmolarity, as damages to cell structures usually lead to cell death and different osmolar pressures can also cause cell lysis due to swelling or shrinking. Extender solutions usually include egg yolk, glycerol and antioxidants and are made from a basic buffer solution of Tes-TRIS. Some variations of this solution have been used to freeze wild felids semen, as for example for tigers' which has been frozen using a deionized water solution with 20% egg yolk, 11% lactose (for energy supply) and 4% glycerol, or similarly using a basic TEST (Tes-TRIS) plus egg yolk solution with 7.5% glycerol, both reaching similar results in post-thaw cell integrity and motility (Luvoni, 2003). In other studies, an almost identical approach was used with ocelot, tiger and domestic cat gametes, using TEST-egg yolk buffer (TYB) plus 4% glycerol (Stoops, 2007; Cocchia, 2010). Iberian lynx (*Lynx pardinus*) semen has also been successfully frozen using 20% egg yolk and 4% glycerol on a simple TEST buffer (Gañan, 2009). Then, there seems to be a consensus in using TYB plus glycerol for felid semen cryopreservation, though recent studies have been focusing on substituting it for soy lecithin-based cryopreservation medium (SOY) as it is more easily obtainable and avoids the use of animal protein, which is usually more prone to bacterial contamination (Vansandt, 2016). Finally, further research is necessary to determine the most adequate media for freezing different species germplasms according to their physiologic singularities and specific requirements.

4.6 Ex- and in-situ preservation of neotropical felids

ARTs ultimate focus is promoting endangered species conservation, such as neotropical felids, specially through captive reproduction of such animals but also by allowing the exchange of genetic material between these and wild populations (Silva, 2019; Swanson, 2007). Such relevance is highlighted by The Species Survival Plans, designed by North American zoos, which aim at maintaining in captivity, over 50 to 100 years, at least more than 90% of the genetic variability lost in nature through the

introduction of founder genes from wild donors (Swanson, 2006). Increasing heterozygosity is necessary in order avoid losses in genetic variability, which may result in perpetuation of deleterious traits or conditions such as teratospermia, as discussed before (Silva, 2019).

In this scenario, zoos and private conservation organizations play a crucial role, primarily by sheltering individuals which are not suitable to reintroduction into the wild but also by conducting reproduction programs, either assisted or natural. The relevance of such entities - as well as their valuable staff and collaborators - in the preservation of wildlife cannot be understated therefore strong partnerships must be established, usually including training and technology transference among institutions (Swanson, 2004). Sharing knowledge and experience and investing in the formation of future researchers is a key factor in increasing assisted reproduction efforts success rates.

5 CONCLUSION

Neotropical felids have faced significant decrease of their populations in the wild. Besides preventing poaching and habitat loss, assisted reproduction can also be used to help restore these species numbers in nature and safeguard their continuity.

Despite inhabiting the whole extent of Latin America, not much is known about these animals reproductive physiology, except for the large representants like the jaguar and the puma. This seems to happen because such animals are commonly found in captivity, either in zoos or private conservational institutions, and can also be easily found in the wild. In addition to that, these are charismatic, fascinating beings, what typically helps obtaining funds for their research.

On the other hand, less conspicuous species like the Andean cat and the kodkod are hardly found either in natural or captive environments, which implies in a total lack of information regarding their reproductive characteristics.

Available information is frequently divergent, as demonstrated in this review. Methods used for testicular measurements or sperm collection for example are often varying and not fully documented, which impede proper comparisons. Standardized procedures should be defined and used to enable better contrasting of collected data.

Cryopreservation of germplasm and ARTs are promising tools in helping preserve wild felids along with collaboration between zoos and conservation organizations. Much has been done in the field, many times culminating in cubs successfully produced through artificial insemination, but better results can be accomplished if species' reproductive biology is fully known. Therefore the need for further investigation on their reproductive physiology can never be overemphasized, and deeper, more comprehensive studies must be conducted in order to empower the conservation of such magnificent animals.

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