ACINONYX JUBATUS REPRODUCTION: FACTORS AFFECTING CUB MORTALITY AND CHEETAH LONGEVITY

By

Gabrielle DeLeon

April, 2021

Allegheny College

Department of Biology

ACINONYX JUBATUS REPRODUCTION: FACTORS AFFECTING CUB MORTALITY AND CHEETAH LONGEVITY

By

Gabrielle DeLeon

April, 2021

A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____***Gabrielle DeLeon***_____

Pledge

Approval by the Senior Thesis Committee:

_____

Dr. Mumme

_____

Dr. Bradshaw-Wilson

ABSTRACT

The cheetah (*Acinonyx jubatus*) is the last surviving species of the *Acinonyx* genus. Known to have low genetic diversity due to two population bottlenecks in their history, they now suffer from low reproduction rates. Both captive and wild populations of cheetahs are known to experience high levels of cub mortality during the first year of life. The international cheetah studbook offers information about births and deaths of captive cheetahs every year. The goal of my study was to unveil issues surrounding low reproduction rates in cheetahs, cub mortality rates, and cheetah lifespan. Overall cub survival rates increased significantly with dam age, however the probability of surviving the first year decreased as sire age increased. Litter size had a complex effect on cub mortality, as cubs from intermediate-sized litters were more likely to survive the first year than cubs from small or large litters. Location of birth also played a role in cub mortality as cheetah breeding facilities in Africa proved to be successful in lowering the rate of death in the first year of life. Further study of these factors in controlled environments could contribute to an improved understanding of the complex issue of cub mortality in cheetahs and the factors affecting the success rate of cheetah reproduction.

INTRODUCTION

The cheetah (*Acinonyx jubatus*) is the last surviving species of the *Acinonyx* genus and is considered unique in both anatomy and behavior from other members in the family Felidae (Marker & O'Brien, 1989). Cheetahs, which are the fastest land mammal, are slowly dying out due to low genetic variation from two population bottlenecks throughout history. Populations of cheetahs made a comeback after the first two bottlenecks, but it now appears as though this

species may quickly be approaching its third bottleneck in spite of proactive reproductive efforts from biologists and veterinarians across the globe.

Genetic bottlenecks can occur due to environmental events that reduce the effective population size, thereby reducing genetic variability. While there is no evidence of great genetic variability in the cheetahs before the first bottleneck, even a drastic reduction in population should be able to conserve 75% of the original genetic variance (Merola, 1994). When compared to other big cats and mammals, cheetahs have displayed between 90% and 99% less overall genetic diversity (O'Brien et al., 2017). This low genetic diversity leaves them more susceptible to illness and is thought to play a role in poor reproductive success in both captivity and the wild. Low genetic variability is not only a repercussion of the bottlenecks cheetahs have suffered in the past, but is also a result of pervasive inbreeding (Merola, 1994).

Inbreeding depression is a term associated with a decrease in biological fitness as a result of common ancestry between mates. Evidence of this condition in cheetahs from captive populations include symptoms such as reduced litter size, increased susceptibility to disease, and infertility (Merola, 1994; Hedrick & Kalinowski, 2000). More directly, homozygosity, the possession of two identical alleles or specific genes in an individual, and spermatozoa abnormalities, have been identified as symptoms of inbreeding depression in captive populations (Merola, 1994). Homozygosity has been widely accepted as the reason for the difficulty in successful births (Merola, 1994). For males, inbreeding has been identified as a cause of high levels of spermatozoa abnormalities in characterizing the ejaculate (Merola, 1994). This common factor influenced scientists to speculate that the large number of abnormal spermatozoa produced by male cheetahs are caused by genomic homozygosity (Wildt et al., 1983).

In North American captive populations, the lack of success in cheetah reproduction has been well documented. Between the 1950's and 1980's, cheetah pairing success rates were low, rarely exceeding 15% (O'Brien et al., 2017). Data accumulated in the 1990s found that 80% of free-range cheetahs were reproducing, while a study in 2015 found only one third of cheetah pairings in North American zoos are producing offspring (Brown et al., 1996; Koester et al., 2015). Even with the few successful births occurring in zoos, the captive population is hardly sustainable. The few cheetah cubs who were successfully born were hard to keep alive since they had a 30% to 40% higher cub mortality rate than almost all other zoo animals (O'Brien et al., 2017).

To help combat this, management and conservation plans for cheetahs have been established by organizations such as the American Association of Zoos and Aquariums (AZA), and similar organizations around the world. The AZA has implemented a species survival plan (SSP) for cheetahs in hopes of increasing not only the percentage of successful births, but the genetic variability as well ("Association of Zoos and Aquariums" n.d.). Data obtained from North American breeding centers demonstrate that greater than 90% of their successful litters were born off-display from the public since 2003 (Koester et al., 2015). In response, other programs such as the Conservation Centers for Species Survival (C2S2) have launched the Cheetah Sustainability Program (CSP) in partnership with AZA's Cheetah SSP. Their objectives are to build a long-term, healthy cheetah population in captivity while also contributing to the conservation of cheetahs in the wild ("Conservation Center for Species Survival," n.d.). The CSP has formed partnerships with five of the nine cheetah breeding centers in the US, including the Fossil Rim Wildlife Center (TX), Omaha's Henry Doorly Zoo (NE), Smithsonian Conservation Biology Institute (VA), White Oak Conservation (FL), and The Wilds (OH). The

goal of these centers is to have sufficient space for exploring mate choice and enhance the behaviors associated with the mating process ("Conservation Center for Species Survival," n.d.). Space is a crucial element in the breeding centers because cheetahs can be unpredictable in their reproductive success. For instance, in one breeding attempt, two male cheetahs ended up killing two females at the Phoenix Zoo (Caro,1993). A recent study found that AZA's SSP breeding and management plan is in fact minimizing inbreeding while the genetic variability in wild cheetahs continues to decline (Terrell, 2016).

Proper management of cheetahs, both in the wild and in captivity, may be the solution to low reproductive success. A survey comparing the reproductive physiology of cheetahs in captivity versus the wild found no differences in reproductive systems but did find differences between the institutions that housed them (Wielebowski, 1996). The contrast in breeding success and cub mortality rates among facilities draws attention to the different husbandry techniques each one uses (Wielebowski, 1996). However, it is worth remembering that cub mortality is high in both captivity and the wild (Stephen, 1994). In the wild, males are known to reside in groups called coalitions, while females are solitary. Similar behaviors are seen in captivity where dual housing of unrelated females result in complete ovarian activity shutdown, which can be resolved by separation of enclosures (Koester et al., 2017). When it comes to male conspecifics, the female is more likely to accept the male when they are allowed to habituate to the enclosure and acclimate to the male scent previous to meeting (Martin-Wintle et al., 2019). This is a beneficial tactic since the rate of scent marking and vocalizations appear lower in captivity (Caro,1993). However, other studies have found similar findings between captive and free-range cheetahs presuming that the likelihood of conceiving is based on the specific situation of the female in captivity (Laurenson & Caro & Borner, 1992). This data is supported by other studies,

where the data between captive and free-range cheetahs, both male and female, seem to be quite comparable for the vastly different conditions. In fact, one study found “no difference between wild caught and captive held animals in average age, body weight, total testicular volume or sperm motility and characteristics" (Crosier et al., 2007)

This study aims to discover the factors affecting the success rates of cheetah reproduction in the wild and in captivity; more specifically, analyzing which factors contribute to high mortality in the first year of life. I hypothesize that the location of birth and the age of the sire or dam will play a significant role in cub longevity. Data from multiple organizations, including the AZA overseeing captive cheetahs and other organizations monitoring wild cheetahs, was collected and compared in order to discover commonalities and differences. This data is accumulated in the International Cheetah Studbook which registers all cheetahs in the world both captive-born and wild-caught, and includes cheetahs held in both private and public facilities and zoos. The studbook is organized and computerized using the international species information system (species360, 2021). Learning how to observe the behaviors of cheetahs in the wild may help to create more productive breeding conditions for members of this vulnerable species held in captivity. With relatively high reproductive success rates in the wild, it is important to understand the factors that lead to that success, be it behavior, reproductive functions, non-human interaction or temperature.

MATERIALS AND METHODS

The following research uses data gathered from the International Cheetah Studbooks, years 2005 through 2011. The studbook is a collection of data gathered from the AZA species 360, Zoological Information Management System (ZIMS), and from similar programs around the world. I transferred key information obtained from this document into a google sheet including:

sire/dam/offspring ID, sire/dam/offspring date of birth, offspring location of birth, offspring rearing treatment, and offspring date of death. The 2018 and 2019 studbook were used to calculate the age of death for many of the offspring when applicable. The following information was then analyzed using google sheets: sire/dam age when offspring was born, offspring age at death, litter size, and country of birth. The total data consists of 945 offspring from over 294 litters throughout Africa, Asia, Australia, North America and Europe. The cheetah survival data was analyzed using JMP pro 15.0. Non-parametric survival analysis and Cox proportional hazards models were used for survival curves. Data on the probability of surviving the first year was analyzed using logistic regression analysis where cheetah survival was considered a categorical variable (survived the first year or failed to survive the first year).

RESULTS

Some research states that female cheetahs undergo uterine changes around the age of 8 (Crosier et al., 2011), but this is not supported by the data. Females are being bred anywhere between the age of 2 and 12 while the males are bred for an even larger period of time, between the ages of 1 and 15 (Figure 1). Both the mean and variance differ significantly in this figure. While younger sires and dams seem to have less cub mortality, the offspring of older sires and dams tend to have a longer lifespan (Figure 2). However, neither of the chi-squared values were significant.

Litter size was found to be a significant factor in cub survival. It is most common to see litters of 3 to 5 cubs or 1 to 3 cubs (Figure 3A). It is least common to see litters of 5 or more; however, litters of 3-7 cubs appeared to produce cubs with the highest survival (Figure 3B). This factor seemed to be most significant in the first year of life. Results showed that small litters have a greater rate of cub mortality than intermediate litters and large litters (Figure 3B).

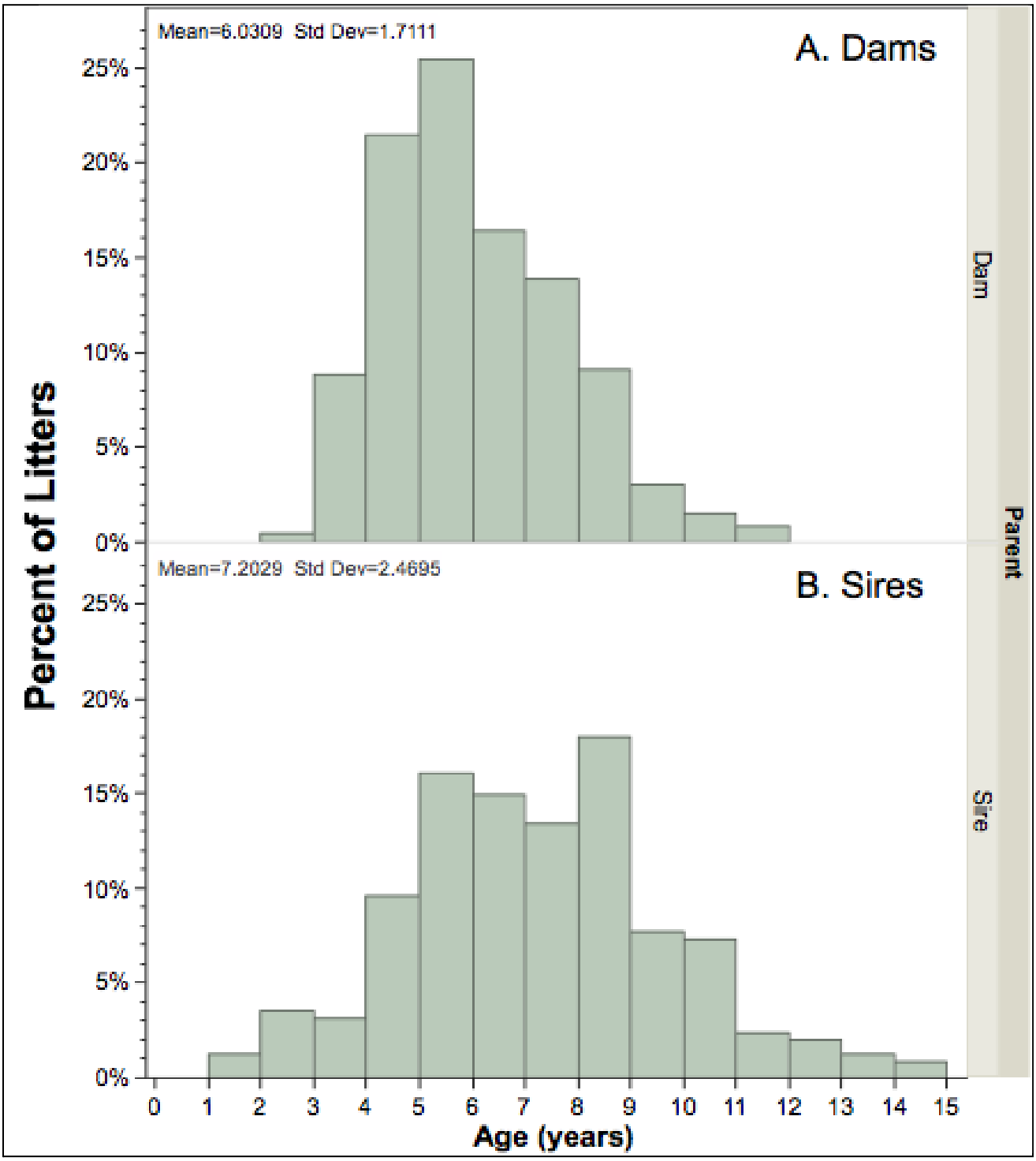


Figure 1: **Distribution of Dam and Sire ages** for 276 and 263 of the 294 litters total litters. Mean: Unequal variance t-test 6.38, $P < 0.0001$. Levene's test for unequal variances $F_{1,537} = 29.50$, $P < 0.0001$.

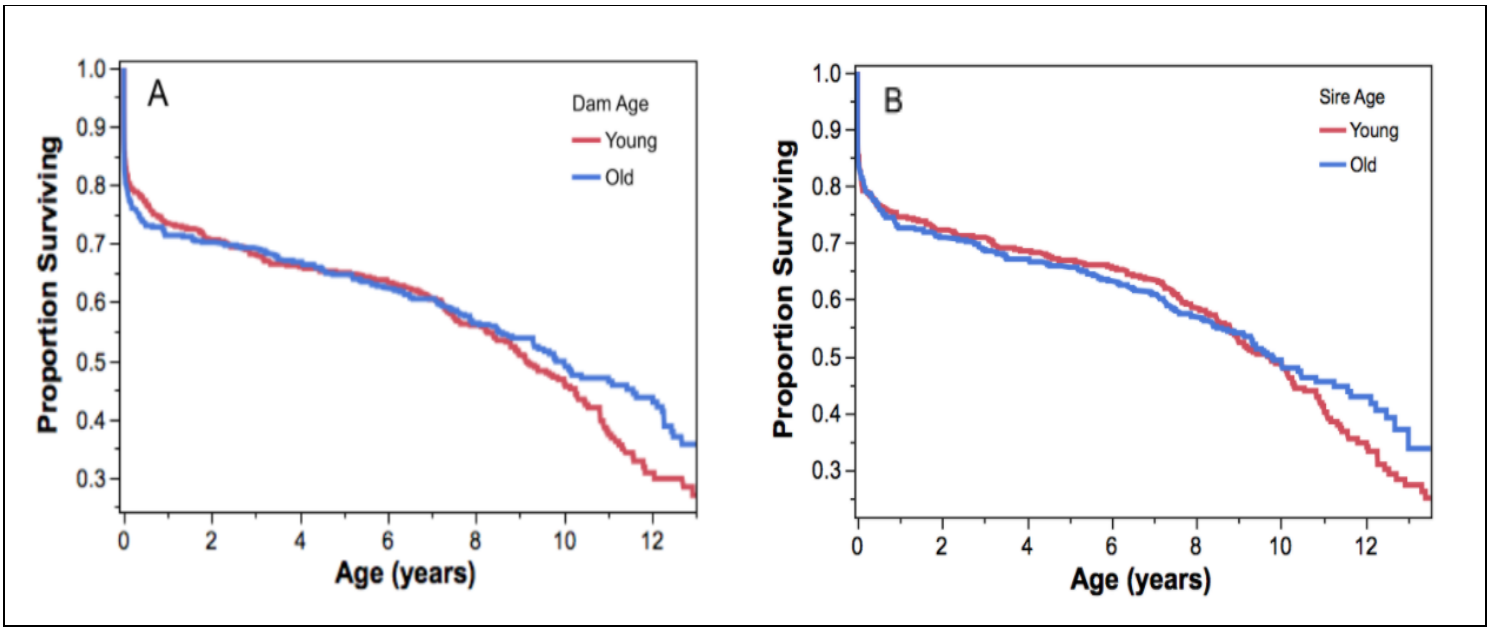


Figure 2: **Effect of dam/sire age on offspring survival.** A) Dam: Cox PH Wald Chi-square = 0.00, P = 0.95 B) Sire: Chi-square =0.00, P = 0.96. Sire age < 7.039 is young. Dam age < 5.849 is young

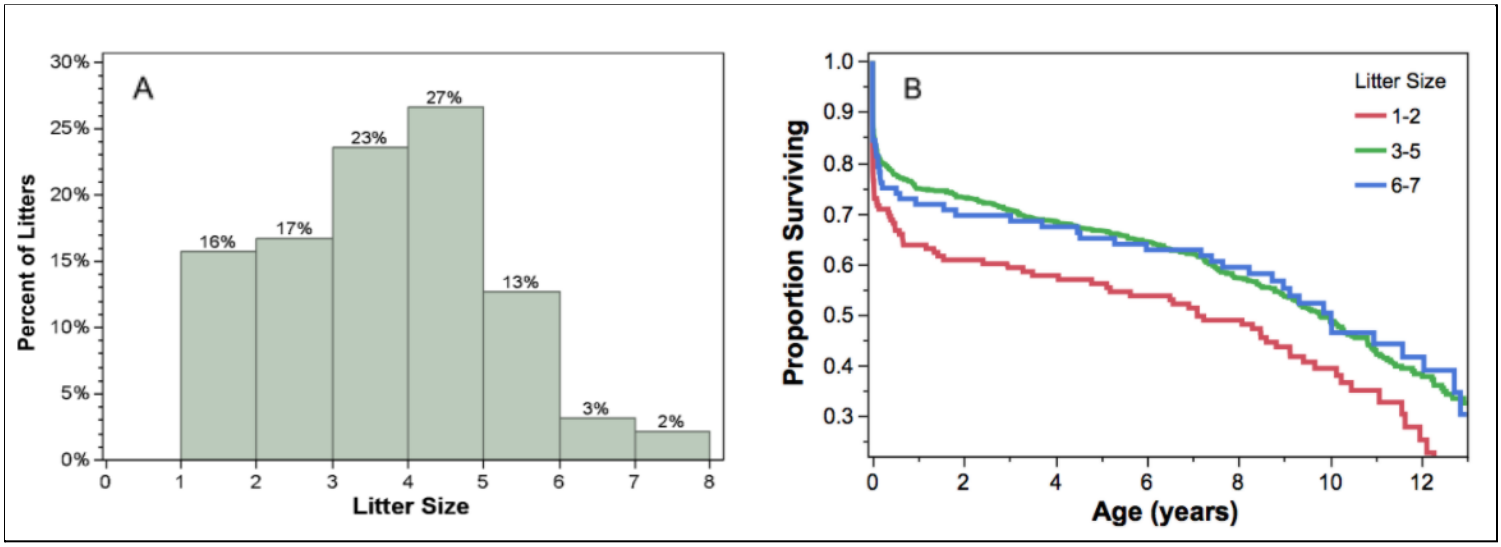


Figure 3: **Litter size.** A) Varies from 1-7, 294 litters total. Mean litter size 3.2, standard deviation 1.5. B) Wald Chi-Square 7.53, df = 2, P = 0.023.

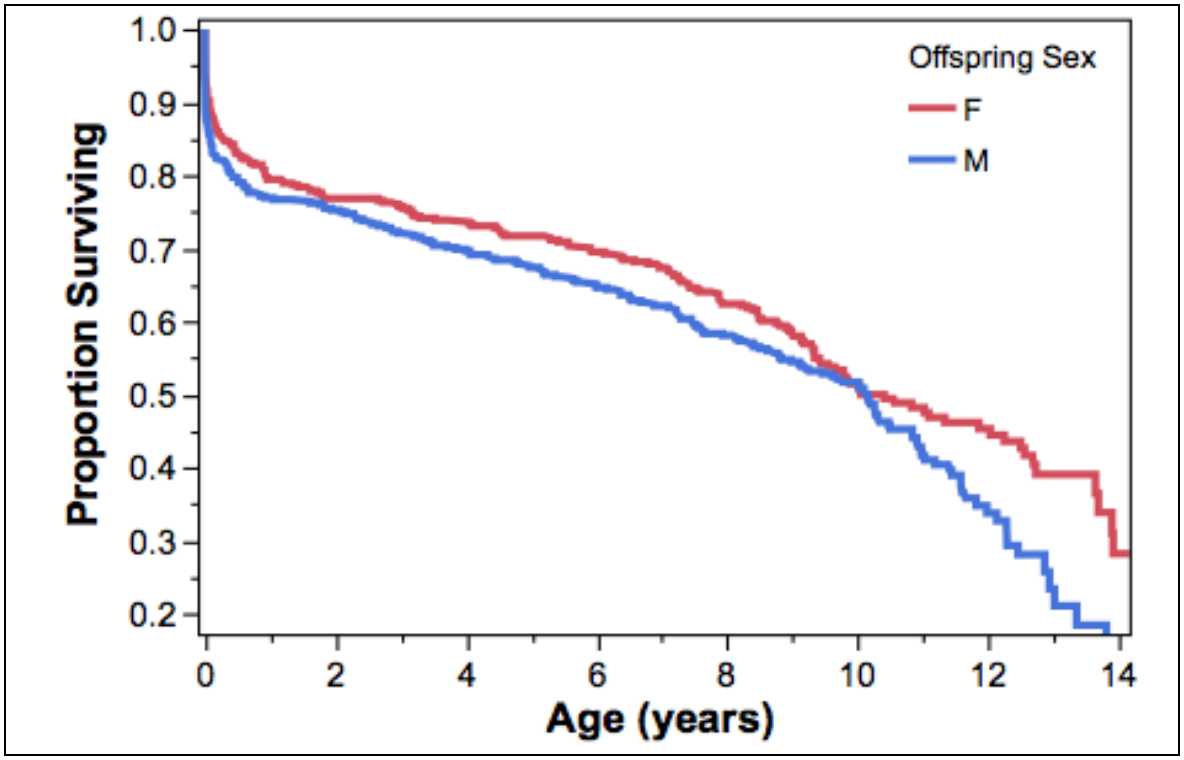


Figure 4: **Sex difference in offspring survival**. Cox Proportional Hazards Model Wald Chi Square 4.25, df =1, P = 0.0392

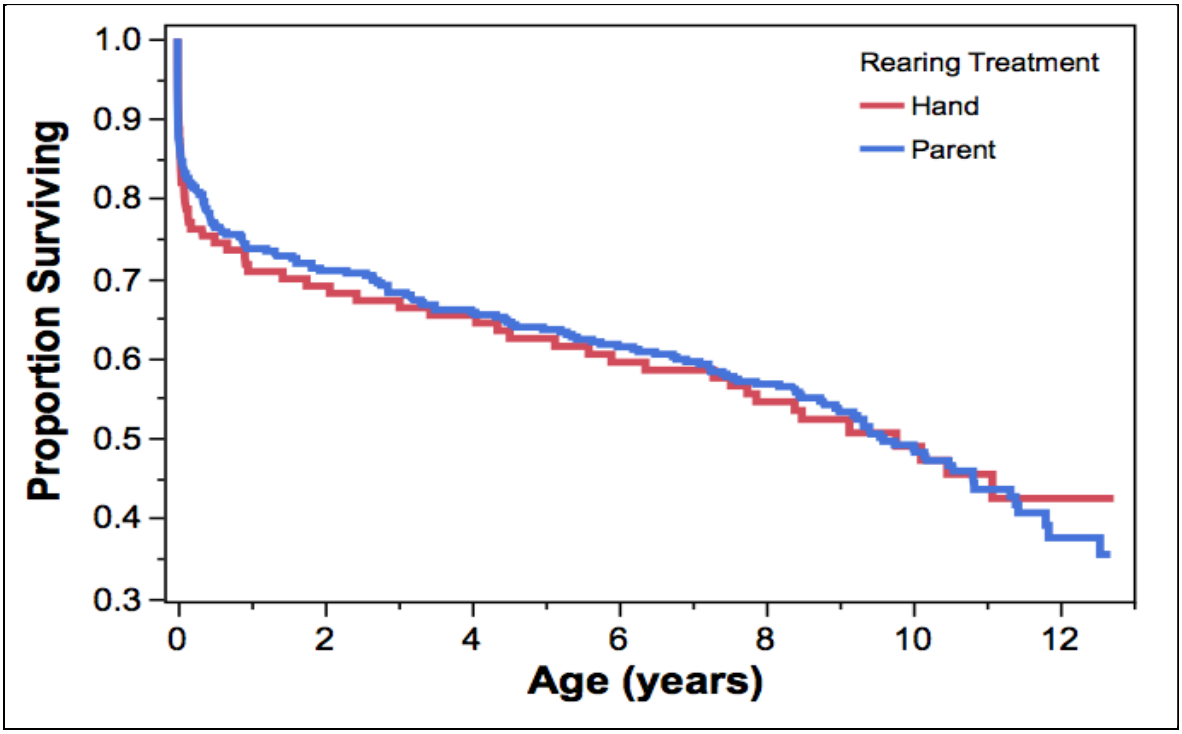


Figure 5: **Rearing treatment (4yrs).** Wald Chi-square = 0.02, P = 0.89

Cub survival was consistently higher for females cubs than male cubs, a result that was statistically significant, yielding a p-value of 0.0392 (Figure 4). Rearing treatment, however, had no significant effect on cub survival, but rearing treatment data were available for only four years of studbook data (Figure 5). Based on the limited data, it seems that whether the cub was hand-raised or raised by its mother has no effect on cub lifespan (Figure 5).

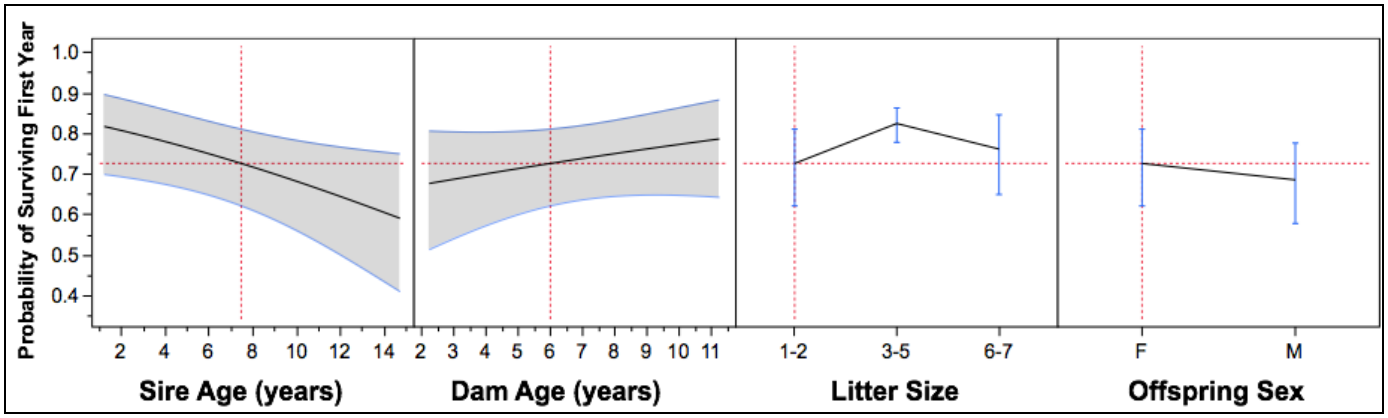


Figure 6: Probability of surviving first year. Sire age, dam age, litter size, offspring sex. Sire age and litter size significant.

Table 1: **A)** Multivariate Cox proportional hazards model of overall cub survival. Cubs from old dams survive better when controlling for the other variables (s age, o sex, and litter size). **B)** Multivariate logistic regression of probability of surviving that first year: Sire age and Litter size significant.

A)

Source	Nparm	DF	Wald ChiSquare	Prob>ChiSq
S age	1	1	1.45127842	0.2283
D age	**1**	**1**	**5.82613247**	**0.0158***
O Sex	1	1	3.26196343	0.0709
Litter Size Category 2	2	2	4.56417218	0.1021

B)

Source	Nparm	DF	Wald ChiSquare	Prob > ChiSquare
S age	**1**	**1**	**4.9215097**	**0.0265***
Litter Size Category 2	**2**	**2**	**6.1758796**	**0.0456***
D age	1	1	1.1577248	0.2819
O Sex	1	1	1.1381434	0.2860

To pursue these factors further, multivariate Cox proportional hazards models and multivariate logistic regression were performed in order to examine overall survival and the probability of surviving the first year, respectively (Figure 6; Table 1). Dam age was positively associated with cub survival when controlling for other variables such as offspring sex and litter size (Table 1). Probability of surviving the first year of life decreased significantly with sire age but was significantly higher for intermediate-sized litters (Table 1B, Figure 6). This means that these factors should be considered when thinking specifically about cub mortality rates. The cubs that statistically have the best chance at survival are part of a medium-sized litter, have a young father, and have an older mother (Figure 6). Once other variables are controlled statistically, offspring sex had no significant effect on either overall survival or probability of surviving the first year of life (Table 1, Figure 6).

Location of birth was one of the most influential factors in cub survival (Figure 7). Cheetah breeding facilities in Africa and North America, respectively, were most successful at keeping their cheetah cubs alive (Figure 7), while Europe, Australia, and Asia struggled with cub mortality and keeping their cheetahs alive to older age (Figure 7). Africa and Europe had the most births during the 7-year period I examined while Australia had the least at 28 total births (Table 2). When comparing all birth years to each other, there was no significant effect of year on cub survival (Figure 8).

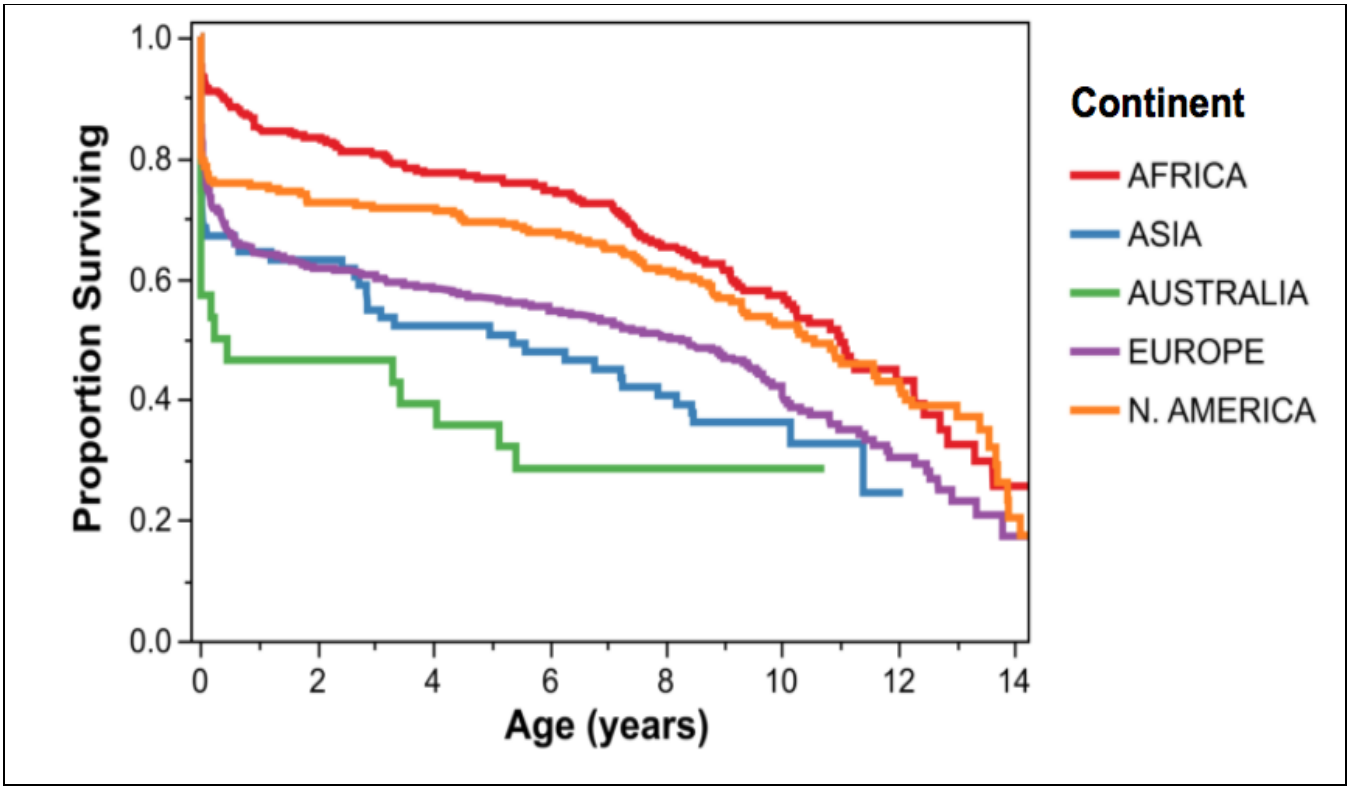


Figure 7: **Proportion Surviving by Continent.**

Table 2: **Birth numbers and mean lifespan by continent.** Failed = death, Censored = alive

Group	Number failed	Number censored	Mean	Std Error
AFRICA	129	172	9.0681	0.30924
ASIA	50	29	5.53852	0.57057
AUSTRALIA	20	8	2.34661	0.48743
EUROPE	197	117	6.92728	0.34704
N. AMERICA	125	94	8.55049	0.4025
Combined	521	420	7.9533	0.19942

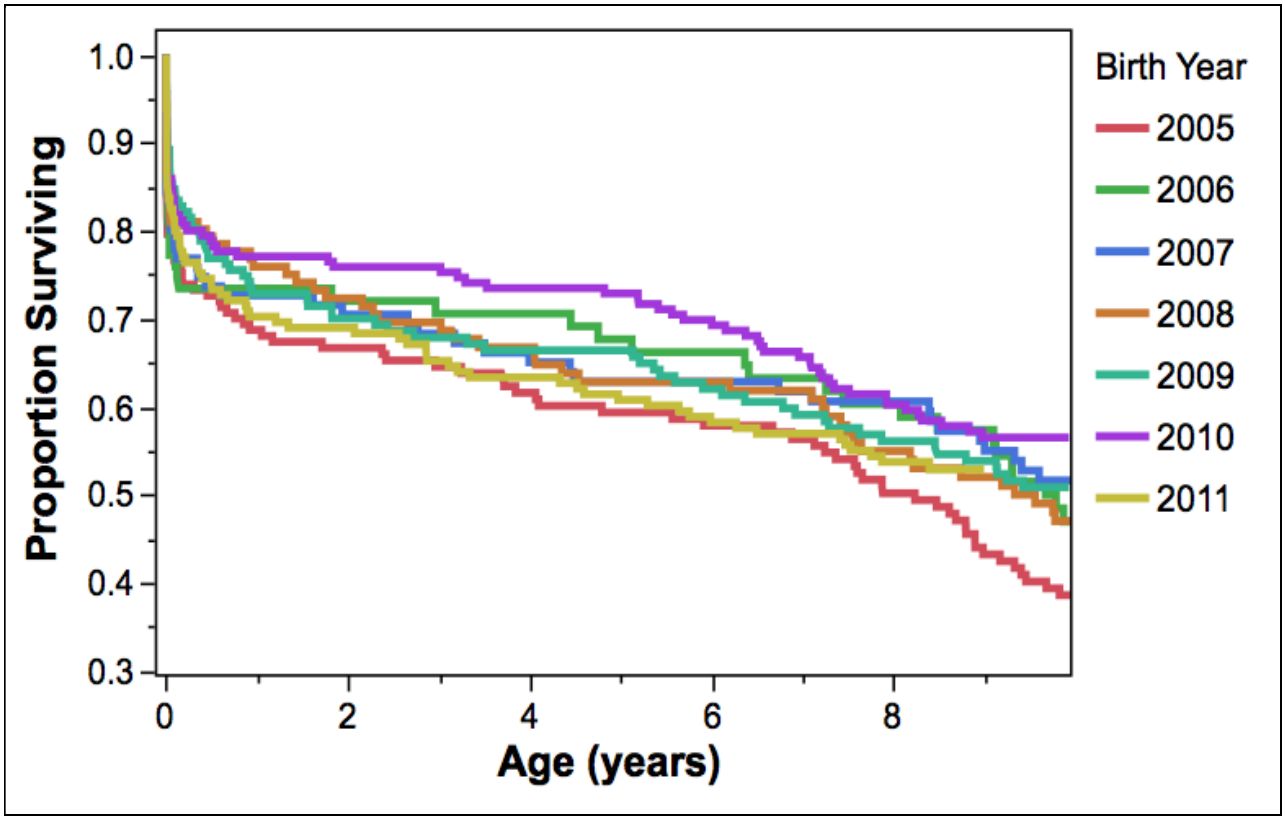


Figure 8: **Birth year survival**. Wald Chi-square 10.23, df = 6, P = 0.12

DISCUSSION

After the statistical analysis of the data one of the most important questions was answered, “Does dam/sire age have an effect on offspring longevity?”. Statistically speaking the age of the parents had no significant effect when directly compared to their offspring's overall lifespan. However, the data raised a number of further questions. When considered individually, neither dam age nor sire age had a significant effect on cheetah overall survival; but when other potentially confounding variables were controlled, dam age was positively associated with overall cub survival and sire age was negatively associated with cub survival during the first year (Figure 6 and Table 1). This may be related to cheetah fertility.

The older the dam the greater the chance of the offspring's overall survival (Figure 6 and Table 1). It is suspected that younger female cheetahs may be less fertile than older females, which directly reflects the conclusion that female fertility increases later in life (Laurenson & Caro & Borner, 1992; Hughes et al., 2002). Due to the stress of cheetahs having a low reproductive rate, fertility becomes a complicated issue of experience versus quality. Do the benefits of having a cheetah who is experienced as a mother and breeding at the age of 10 outweigh the benefits of a younger dam who produces quality cubs up until the age of 7? This question was raised by Pettorelli & Durant (2007), and can be applied to this study. Studies have shown that long-lived females have a higher chance of producing long-lived female offspring, and that polyandrous females avoid the cost of inbreeding (Pettorelli & Durant, 2007; Tregenza & Wedell, 2002). This would explain why cub survival is higher with older dams. The experience of being a mother and giving birth seems to yield extended cub longevity. Also, there may be nutritional benefits passed on from older mothers if their cubs are living longer.

While older dams produce cubs with a greater chance of survival, cubs of older sires are less likely to survive the first year of life. This is important because the application of the mutation accumulation theory helps to explain the difference in outcome between young and old males and females. Females are born with a certain amount of eggs to reproduce, and the quality of these eggs do not decline over time because they are not dividing or mutating. If females are less likely to suffer from inbreeding and genetic mutations then this theory most likely applies to the male population who suffer from sperm mutations. Sperm is different from the females' eggs in the sense that they are produced by constantly dividing cells which can be prone to mutation. This could explain why young sires and older dams tend to produce offspring that are more likely to survive the first year. It is important to point out that the same amount of abnormal

sperm has been measured in young and old populations (Wildt et al., 1993). Therefore, the mutation accumulation theory does not raise concern over an increase in abnormal sperm but rather a decrease in the functionality of the abnormal sperm.

Surviving the first year of life is a significant accomplishment for cheetahs, both in captivity and in the wild, and poses the question of the importance of parental experience versus genetic quality. If quality offspring are the ideal result, how can we combat first-year cub mortality in a population? Little is known about why cheetahs have such a high cub mortality rate in captivity. The multivariate logistic regression suggests that litter size and sire age are two significant factors in surviving the first year of life. The mutation accumulation theory provides a framework for understanding why the cubs of older sires are less likely to survive the first year. Older males are most likely to suffer from decreased sperm quality than the younger males which may play a role in their offspring's genetics and overall health. There are not many studies relating the sire age to the litter size, but interestingly in dogs, the age gap between the sire and the dam actually has an effect on the gender within the litter. For instance when a young sire and an old dam mate, or vice versa, there is a higher percentage of males in the litter (Martins et al., 2019). A smaller percentage of males in the litter occurred when young mated with young or old mated with old (Martins et al., 2019). This is important to consider in the cheetah population because females were seen to have a higher chance of survival than males, although this difference was not statistically significant when other potentially confounding variables were controlled. It is possible that the sires play a greater role in cub mortality because they are the ones passing along more abnormal genes caused by accumulated mutations in the germ-line cells, and these genetic changes could be causing health issues for the cubs. This is a valid theory

as males suffer a little bit more from cub mortality and they receive the Y chromosome from the father (Yatsenko & Turek, 2018).

Rearing treatment also raised concern about whether hand-raised cheetahs were receiving enough proper nutrients in order to live full lives. Data gathered over 4 years (2007, 2008, 2009, and 2011) showed no difference in hand-raised versus mother-reared cubs. Hand-raised cubs did see a slightly lower dip in the first year of life (Figure 5) but this cannot fully be evaluated from a nutritional standpoint without necropsy information. It is most likely that this reduction in sustainability was due to other underlying causes. Two themes that should be further researched in cub mortality are the rate of death by infection and death by exhibit mate attacks.

One common concept throughout the data analysis was that litter sizes above 3 cubs were more likely to live longer than 1 or 2 cub litters. And, litters of 3 to 5 cubs were 10% more likely to survive the first year of life. There has been little research on litter size in cheetahs and it is difficult to analyze the factors involved in litter size. This could be fertility and sperm related, nutrition related, or weather/environment related (Gigliotti et al., 2019). It is possible that small litter sizes indicate a higher instance of health issues while larger litters indicate healthier mothers. This may be why the cubs are more likely to survive when part of larger litters (Figure 3B). Some sense can be made of this from the wild population, as some of the data from the studbook was obtained from wild cheetah parents in Africa. As expected, the wild cheetah cub population suffers most from predation from other species and human interactions (Broekhuis, 2018). However, further research would be needed on the wild population as well as on the correlation between litter size and the season of birth.

One of the most interesting findings of my study is the extreme differences in proportion of cheetahs surviving by continent. Europe, Africa and North America had the most births over a

seven-year period, respectively. While Europe had the most data to pull from they came in 3rd and Africa came in first in cub survival. But, it's not the trend of the survivorship curve that is unusual; it is the immediate decline in the first year of life. Once again, cub mortality is at play in each continent. Africa only lost 10-15% of the offspring in the first year of life, while N. America lost about 20% and Europe lost almost 27%. Specific differences in husbandry are hard to identify as each individual zoo represented in the data would need to be observed and their techniques compared. Some common issues that may play a part in breeding success are partner pairings, exhibit, nutrition, stress and human-interaction. Most if not all of the participating zoos and private breeders should be held to certain standards, ensuring adequate nutritional guidelines. Exhibits may be too small due to area restrictions and increased levels of animal stress may occur. Human interactions may also cause increased stress as cheetahs are known to be more viable in more isolated enclosures away from the public during estrus. Isolation may sometimes be hard to achieve and perhaps that may be why Africa and North America perform well with open savanna and large cheetah breeding centers on those continents. It should also be noted that depending on the environment in which some of the animals are kept, that some cubs may have already died or been attacked before being able to count the number of cubs in the litter. There are many factors that play a role in cheetah reproduction and it is essential that more research is done in order to make sure the population is more sustainable for future generations.

CONCLUSION

Even though research on cheetah reproduction continues to be a hot topic, it is still difficult to determine the causation of low reproductive success and high mortality during the first year of life. It is evident that cub mortality plays a big role against the effort to make the cheetah populations self-sustaining. Many organizations like the AZA and similar ones abroad are vital

to this continued effort. While the cheetah studbook contains valuable information on cheetah reproduction, it seems that more specific information is needed in order to assist this species. Litter size and sire age is a topic that should be studied further with regard to cub mortality. In addition, the effects of litter size on cheetah lifespan should be investigated within both the captive and free-range populations. It is evident that Africa has the best record on cub survivability and it may be beneficial to explore the unique characteristics of their breeding habitats and programs. Further research and development of successful cheetah reproduction programs will ensure the continued survivability of this species for generations to come.

LITERATURE CITED

Broekhuis, F. (2018). Natural and anthropogenic drivers of cub recruitment in a large carnivore. *Ecology and Evolution, 8*(13), 6748-6755.

Brown, J. L., Wildt, D. E., Wielebnowski, N., Goodrowe, K. L., Graham, L. H., Wells, S., & Howard, J. G. (1996). Reproductive activity in captive female cheetahs (*Acinonyx jubatus*) assessed by faecal steroids. *Journal of Reproduction and Fertility, 106*(2), 337-346.

Caro, T. M. (1993). Behavioral solutions to breeding cheetahs in captivity: Insights from the wild. *Zoo Biology, 12*(1), 19-30.

Cheetah (*Acinonyx jubatus*), AZA Species Survival Plan, & Yellow Program. *Population analysis & breeding and transfer plan.* https://ams.aza.org/iweb/upload/Cheetah%20-%20Yellow%20SSP%202017%20DRAFT-3c68ea54.pdf

Marker, L., & O'Brien, S. J. (1989). Captive breeding of the cheetah (acinonyx jubatus) in north american zoos (1871–1986). *Zoo Biology, 8*(1), 3-16.

Crosier, A. E., Marker, L., Howard, J., Pukazhenthi, B. S., Henghali, J. N., & Wildt, D. E. (2007). Ejaculate traits in the Namibian cheetah (*Acinonyx jubatus*): Influence of age, season and captivity. *Reproduction, Fertility and Development, 19*(2), 370-382.

Crosier, A. E., Comizzoli, P., Baker, T., Davidson, A., Munson, L., Howard, J., . . . Wildt, D. E. (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.

Gigliotti, L. C., Slotow, R., Hunter, L. T. B., Fattebert, J., Sholto-Douglas, C., & Jachowski, D. S. (2019). Context dependency of top-down, bottom-up and density-dependent influences on cheetah demography. *Journal of Animal Ecology, 89*(2), 449-459.

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics, 31*(1), 139-162.

Hughes, K. A., Alipaz, J. A., Drnevich, J. M., & Reynolds, R. M. (2002). A test of evolutionary theories of aging. *Proceedings of the National Academy of Sciences of the United States of America, 99*(22), 14286-14291.

Koester, D. C., Freeman, E. W., Brown, J. L., Wildt, D. E., Terrell, K. A., Franklin, A. D., & Crosier, A. E. (2015). Motile sperm output by male cheetahs (*Acinonyx jubatus*) managed ex situ is influenced by public exposure and number of care-givers. *PloS One, 10*(9), e0135847.

Koester, D. C., Wildt, D. E., Brown, J. L., Meeks, K., & Crosier, A. E. (2017). Public exposure and number of conspecifics have no influence on ovarian and adrenal activity in the cheetah (*Acinonyx jubatus*). *General and Comparative Endocrinology, 243*, 120-129.

Laurenson, M. K., Caro, T., & Borner, M. (1992). Patterns of female reproduction in wild cheetahs: Implications for conservation. *Research and Exploration, 8*(1), 64-75.

Martin‐Wintle, M. S., Wintle, N. J. P., Díez‐León, M., Swaisgood, R. R., & Asa, C. S. (2019). Improving the sustainability of ex situ populations with mate choice. *Zoo Biology, 38*(1), 119-132.

Martins, A. C. L., Vaz, M. A., Macedo, M. M., Santos, R. L., Galdino, C. A. B., Wenceslau, R. R., & Valle, G. R. (2019). Maternal age, paternal age, and litter size interact to affect the offspring sex ratio of german shepherd dogs. *Theriogenology, 135*, 169-173.

Michele Merola. (1994). A reassessment of homozygosity and the case for inbreeding depression in the cheetah, *Acinonyx jubatus*: Implications for conservation. *Conservation Biology, 8*(4), 961-971.

O'Brien, S. J., Johnson, W. E., Driscoll, C. A., Dobrynin, P., & Marker, L. (2017). Conservation genetics of the cheetah: Lessons learned and new opportunities. *The Journal of Heredity, 108*(6), 671-677.

Pettorelli, N., & Durant, S. M. (2007). Longevity in cheetahs: The key to success? *Oikos, 116*(11), 1879-1886.

Species 360 (2021). https://www.species360.org/

Stephen J. O'Brien. (1994). The cheetah's conservation controversy. *Conservation Biology, 8*(4), 1153-1155.

Terrell, K. A., Crosier, A. E., Wildt, D. E., O'Brien, S. J., Anthony, N. M., Marker, L., & Johnson, W. E. (2016). Continued decline in genetic diversity among wild cheetahs (*Acinonyx jubatus*) without further loss of semen quality. *Biological Conservation, 200*, 192-199.

Tregenza, T., & Wedell, N. (2002). Polyandrous females avoid costs of inbreeding. *Nature, 415*(6867), 71-73.

Wielebnowski, N. (1996). Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. *Zoo Biology, 15*(4), 353-369.

Wildt, D. E., Brown, J. L., Bush, M., Barone, M. A., Cooper, K. A., Grisham, J., & Howard, J. G. (1993). Reproductive status of cheetahs (*Acinonyx jubatus*) in North American zoos: The benefits of physiological surveys for strategic planning. *Zoo Biology, 12*(1), 45-80.

Wildt, D. E., Bush, M., Howard, J. G., O'Brien, S. J., Meltzer, D., Van Dyk, A., . . . Brand, D. J. (1983). Unique seminal quality in the south african cheetah and a comparative evaluation in the domestic cat. *Biology of Reproduction, 29*(4), 1019-1025.

Yatsenko, A. N., & Turek, P. J. (2018). Reproductive genetics and the aging male. *Journal of Assisted Reproduction and Genetics, 35*(6), 933-941.