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Effects of Inbreeding on Survival of Captive Bengal Tigers (*Panthera tigris tigris*) in Lahore Zoo

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ABSTRACT

The risk of inbreeding within captive tiger populations is a fundamental problem in conservation of wildlife. **Objective:** To determined high levels of inbreeding can result in reduced genetic variation, low reproductive performance, increased cub mortality and reduced immune competence. Lahore Zoological Gardens famous for its tiger exhibits. The current study has been palnned to understand the inbreeding in Bengal tigers (*Panthera tigris tigris*) at Lahore due to the production of offspring among similar individuals of the same families without change in blood line. Stillbirths and rickets are an inbreeding issue for tigers at Lahore Zoo. **Methods:** The issue of inbreeding was highlighted in 2008. From 2008 to 2020, repeated cases of inbreeding have occurred. **Results:** Among the 19 new-born cubs, 9 were male and 10 were female. The normal birth rate for cubs was 63% and stillbirth was 37%. The percentage of white-colored cubs was 72%. The newborn tigers suffered from various conditions such as autolysis, squint eyes, blue eyes, white color, malformation, and infection. Because of this, tiger breeding was banned in 2010. In 2018 and 2022 new tigers was imported to replace the blood line. A pair of them produced four cubs, all of which died at the age of 11 weeks. **Conclusions:** Based on this, it was recommended that the genetic analysis of the existing tigers at Lahore Zoo and at different captive sites in the province be performed. Apart from that, revision of the collection plan, blood line change and artificial insemination are also recommended.

INTRODUCTION

Tigers are large carnivores. An iconic and endearing endangered animal, the tiger (*Panthera tigris*) formerly ranged over 70 degrees of latitude in Asia [1]. There are thought to be 2,154-3,159 tigers left, which is fewer than 6% of their range from 1900 AD [2]. The flagship species of Asian forested ecosystems, tigers (*Panthera tigris*), have lost approximately 40% of their territory in the last 20 years [3]. The tiger faces threats to its continued existence, putting it among the endangered species. A lack of genetic variety in a population can lead to a species extinction [4]. Naturally, tiger breeding in zoos will happen if the population needs more tigers. According to genetic variety is a crucial factor in maintaining the risk of extinction [5]. However, inbreeding occurs because zoo populations of tigers breed among themselves to ensure the species survival. Once again, homozygosity rises as a result of inbreeding-induced genetic diversity loss. Homozygosity is the dominant allele for the most harmful characteristics in

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populations. Consequently, the phenotypic value of a trait declines due to inbreeding depression [6-7]. Zoos and wildlife parks are essential to the conservation of this species in addition to in situ initiatives. It's thought that there are currently more tigers kept in captivity than there are in the wild, including zoos and circuses [8]. The detrimental effects of inbreeding on tiger's ability to survive could result in their extinction. Planned breeding should be done to preserve tigers, taking great care to prevent mother-son mating, father-daughter, and sibling mating. Among the other detrimental effects of inbreeding on reproduction and survival (inbreeding depression) is one of the primary genetic concerns in conservation biology [9]. Because inbreeding increases the frequency of the unfavorable homozygous genotype, selection against deleterious genes increases. As a result of which a population can be 'purged' of its mutational burden [10-12]. The Lahore Zoo has seen ups and downs throughout the years, but despite this, it has continued to be a significant institution in the nation, participating in public awareness campaigns, animal relocations, and a variety of breeding programs. Since its inception, the oldest lion and tiger house has been its primary attraction. The number of lions and tigers was on the rise, but due to continuous breeding within the same blood line, inbreeding became a significant issue. For better ex-situ conservation.

The current study was designed to understand the issue of inbreeding and its impact on ecologically important carnivore species.

METHODS

The study site was Lahore Zoological Gardens, Lahore and study animals were Bengal Tigers (*Panthera tigris*). The study is presenting 12 years data of inbreeding in Bengal Tigers.

Import of Bengal Tigers (Panthera tigris) to Lahore Zoo

In 1971 and 1975, a total of 03 Bengal Tigers (Panthera tigris tigris) brown in color were imported to Lahore Zoo. A pair of them died and the only one left was a female named Mary. Later, in 1982 and 1984, two pairs of tigers were imported, of which two were males and two were females. So the total number of tigers was 2:3, all brown. The white tigress, namely Bush, was imported in 1991. Thus, the total number of tigers was 6, of which 2 were males and 4 were females. As per the records collected from the Lahore Zoo stock registers, these six tigers contributed to the present stock of big cats in all Punjab Wildlife Zoos and Parks. The most successful breeding pair was the White Tigress Bush and Brown Tiger Leo. They breed so successfully, but gradually the signs of inbreeding started to appear, like stillbirths and rickets, which were an inbreeding issue in tigers at Lahore Zoo.

Bengal Tiger Stock Position in 2008

At the time of the research work, there were two males and three females present at Lahore Zoo. The details and local names were as under in table 1.

Table 1: Detail of Stock Position (2008)

S.No.	Gender	Number	Color	Local Name
1.	Male	01	White	Sam
2.	Male	01	Brown	Jumbo
3.	Female	01	Brown	Rozi
4.	Female	01	White	Jati
5.	Female	01	White	Mohni

First Inbreeding Case Report (2008)

Five cubs (3:2) were born to mother Rozi and father Jumbo, who happen to be the progeny of the same parents, Bush and Leo. Out of these cubs, three were stillborn and the normal cubs died the same day (Figure 1a and 1b).

Figure 1(a): Stillbirth

Figure 1(b): Normal

Second Inbreeding Case Report (2009)

Again in 2009, four (2:2) healthy cubs were born to Rozi and Jumbo. One male brown cub had paraplegia at 3.5 months of age [(figure 2(a) and 2(b)]. Later, all died one by one within six months of their age.

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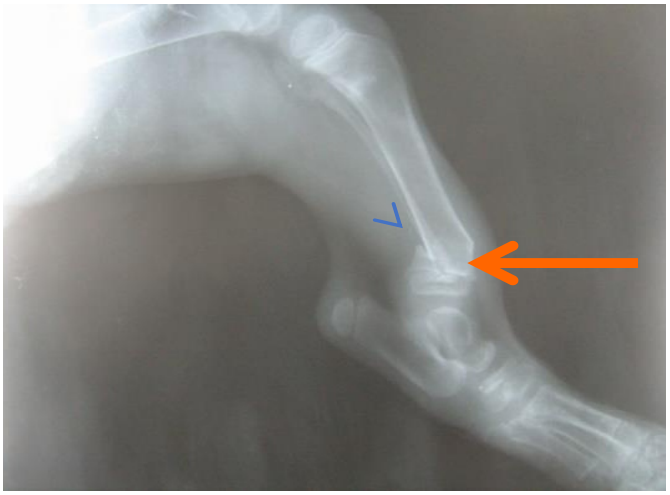

Figure-2(a): Radiograph of Fractures

Figure-2(b): Cub with Displaced Digits

Third Inbreeding Case Report 2010

In 2010, Mother Rozi gave birth to four more cubs. But this time his father was Sam (the White Tiger), who was also from the same bloodline as Bush and Leo (Figure 3b). Two of the born cubs were stillborn. Of the two live born cubs, the white female cub died on the same day, while the other white cub, also named Zona, was healthy and feeding by her mother(Figure 3a).

Figure 3(a): Rozi with Zona

Figure 3(b): Sam

Forth Inbreeding Case Report 2010

On July 30, 2010, Rozi and Sam crossed paths again. Calcium supplements were given to the mother. Prolactin hormone had also been given to increase milk production. But it was all in vain, as the tigress delivered three premature cubs for her fourth breeding on October 29, 2010 (figure 4 a, b).

Figure4(a): Premature White Male

Figure4(b): Premature Birth

Decision by Zoo Management

The Zoo Management banned breeding in the existing stock of big cats until the import of new pairs with changed blood lines in 2010.

Import of New Tigers

Lahore Zoo's management has always tried its best to

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improve captive wildlife conservation and welfare. To keep it in view, in 2018, eight tigers were imported from the UAE. Among them, 3 were brown tigers and 5 were white tigers. In brown tigers, there were 2 males and 1 female. In white tigers, 3 were males and 2 were females. These tigers were imported to produce healthy ones. These tigers were paired, and in 2020, a pair of white tigers gave birth to four tiger cubs.

Fifth Mortality Case Report 2020

The white tiger pair gave birth to four tiger cubs in November 2020. All four tiger cubs were on hand rearing, and they were unable to survive and died one after another within 11weeks, from which one of them was suspected of pneumonia and one was suspected of COVID.

RESULTS

First Inbreeding Case Report (2008)

The pedigree in figure 5 is showing the birth details of Rozi and Jumbo (figure 5).

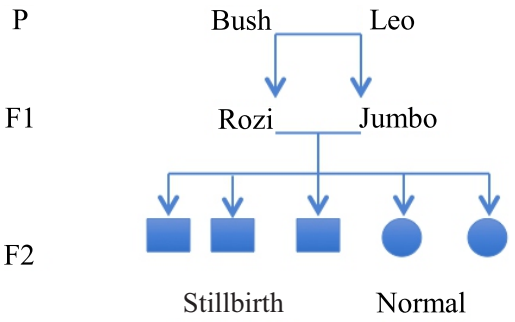

Figure 5: Flowchart of First Inbreeding Case Report 2008

Among the five cubs 3 were males (two white + one brown)] stillbirth and two brown females were normal (table 2).

Table 2: Color and Gender of 2008 Birth

S.No.	Cubs	Color	Gender	Birth Status
1	1	White	Male	Stillbirth
2	2	White	Male	Stillbirth
3	3	Brown	Male	Stillbirth
4	4	Brown	Female	Normal
5	5	Brown	Female	Normal

Second Inbreeding Case Report (2009)

The Rozi and Jumbo produced 4 cubs and all were normal at the time of the birth (figure 6).

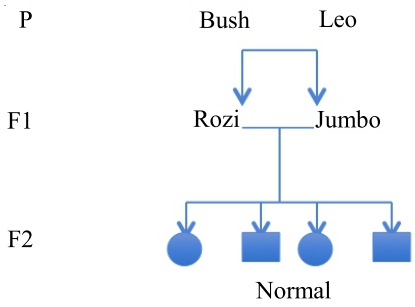

Figure 6: Flowchart of Second Inbreeding Case Report 2009

Among males one was white and other was brown while females were all brown (table 3).

Table 3: Color and Gender of 2009 Birth

S.No.	Cubs	Color	Gender	Birth Status
1	1	White	Male	Normal
2	2	Brown	Male	Normal
3	3	Brown	Female	Normal
4	4	Brown	Female	Normal

Third Inbreeding Case Report 2010

The birth of 2010 produced a brown male and a white female, were stillborn (figure 7).

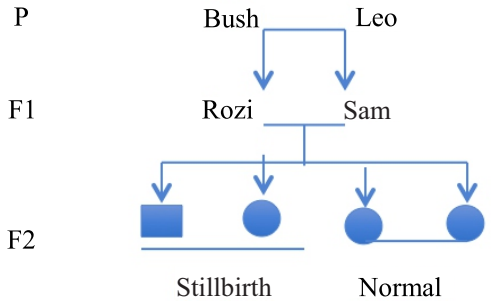

Figure 7: Flowchart of Third Inbreeding Case Report 2010

Of the two live born cubs, the white female cub died on the same day, while the other white cub, also named Zona (table 4).

Table 4: Color and Gender of 2010 Birth

S.No.	Cubs	Color	Gender	Birth Status
1	1	Brown	Male	Stillbirth
2	2	White	Female	Stillbirth
3	3	White	Female	Normal
4	4	White	Female	Normal

Postmortem Findings

The stillborn cub's postmortem findings suggest autolysis, malformation, and infection. While the live born brown cub was undersized and emaciated, a small amount of clotted blood was present in both ventricles. The stomach was distended and swollen due to internal pressure and gas and contained about 10 ml of clear serous fluid, which is a pale-yellow liquid found between organs and membranes enclosing them (figure 8). Acute hemorrhagic gastroenteritis (AHDS) was diagnosed.

Figure 8: Macerated and Normal Cubs Postmortem

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Health of Zona

Zona was on mother feed. On May 6, 2010, it was observed that the cub was losing weight and had not opened her eyes even on the 8th day of his birth. Upon examination, it was discovered that the mother was not producing milk, leading to the hand-rearing of the cub on imported Esbalic and KMR formula milk from the USA. She opened her eyes within 3 days, and the weight gain was proper [figure 9(a), 9(b), and 9(c)]. But Zona was paralyzed on August 23, 2010 at the age of four months [figure 9(d)].

Figure 9: Health of Zona

Treatment

Zona received the most sunlight and supportive therapy. Zona's meat consumption was good until the last three days, when she was forcefully fed. Cub began to walk when the fracture began to heal, and continued to do so until she developed a new fracture.

Complications

The Zona was reluctant to walk and had an abscess due to fracture (figure 10).

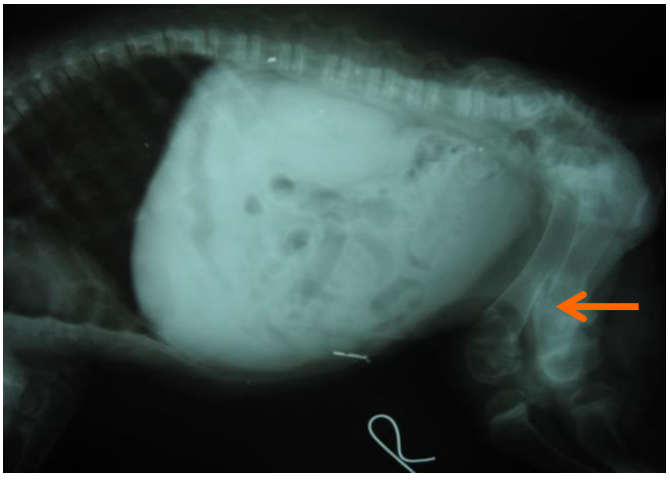

Figure 10: Radiographs Showing Multiple Fractures

Diagnosis, Death and Postmortem

Zona was diagnosed with congenital rickets (born with clinical features of rickets). She had multiple fractures, a potbelly (overweight), squinted eyes (eyes open in different directions), blue eyes, and white eyes. Zona died on September 26, 2010. Professionals conducted the postmortem of Zona on the same day. The postmortem findings suggested congestion in the lungs, an enlarged kidney (figure 11), and a callus and callus between the 8th and 9th ribs (figure 12), which revealed a 3-week-old fracture, hemorrhage (loss of blood) in the hind limb, multiple fractures and rickets.

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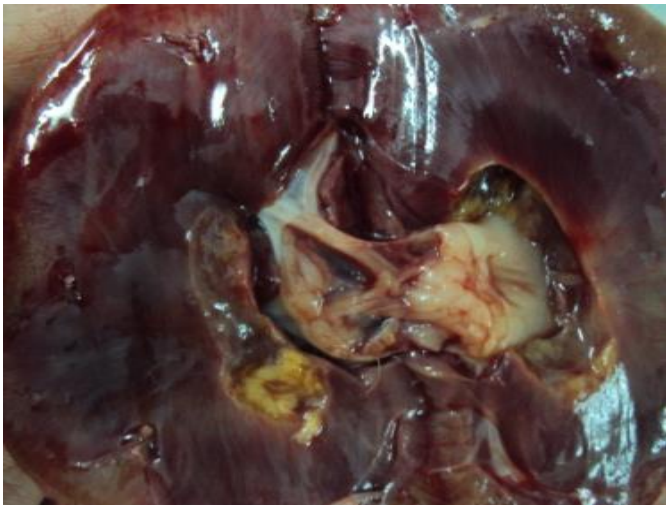

Figure 11: Enlarged Kidney

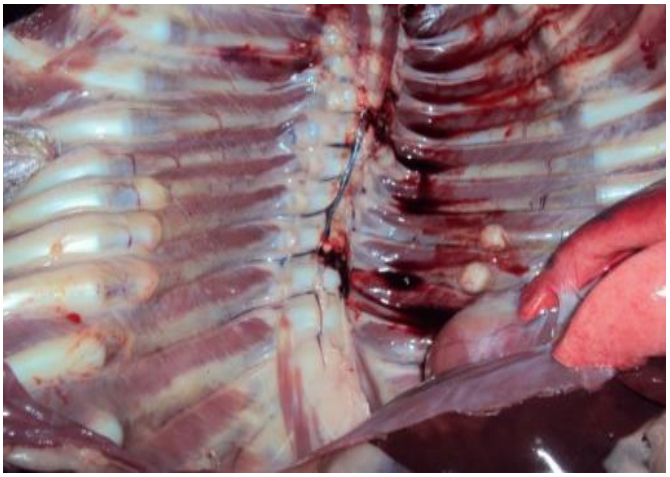

Figure 12: Lesions in Ribs

Table 5: Zona(Birth to Death)

Phase	Date
Birth	29 April 2010
Weight Loss and not Opened Eyes Till	06 May 2010
Eyes were Opened and Proper Weight Gain	09 May 2010
Zona Paralysis	23 August 2010
Death and Postmortem	26 September 2010

Forth Inbreeding Case Report 2010

The pedigree in figure 13 is showing the gender of 2010 birth.

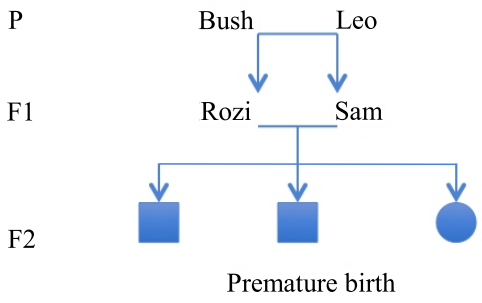

Figure 13: Flowchart of Forth Inbreeding Case Report 2010

According to it the one cub was again a white male, and the color of the other male and female was unidentified (table 6).

Table 6: Color and Gender of 2010 Birth

S.No.	Cubs	Color	Gender	Birth Status
1	1	White	Male	Stillbirth
2	2	Unidentified	Female	Stillbirth
3	3	Unidentified	Female	Stillbirth

Steps of Zoo Management to Control the Inbreeding Issues

The Zoo Management is always ready to take any step for the welfare of captive wild animals. To overcome the inbreeding issues in zoo big cats zoo has imported one White male in 2018 three brown males and two pairs of white tigers in early 2020 respectively. These tigers gave birth to new four tiger cubs from a new bloodline but they did not survive due to some infections mentioned below.

Fifth Mortality Case Report 2020

The cubs borne during 2020 were all white (2:2) two males and two females(figure 14).

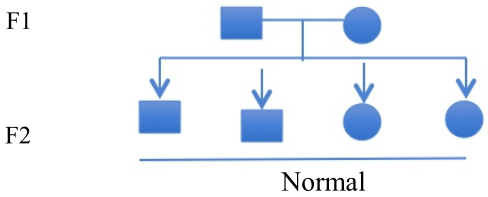

Figure14: Flowchart of Second Inbreeding Case Report 2020

Data regarding gender and race/color for children born in 2020 is unavailable in table 7.

Table 7: Color and Gender of 2020 Birth

S.No.	Cubs	Color	Gender	Birth Status
1	1	White	Male	Normal
2	2	White	Male	Normal
3	3	White	Female	Normal
4	4	White	Female	Normal

Postmortem and Diagnosis

The postmortem revealed the lungs of two cubs were badly damaged and they were suffering from a severe infection. Pathologists were concluding they may have died from COVID-19. The other two have cute enteritis. Hindquarter paralysis was present in all of them. Based on these findings it was not considered as an inbreeding issue because all tigers were recently imported. But the point of concern is the white color of the breeding pair which is due to recessive genes.

Birth Status of Cubs 2008-2020

Figures 14a, b, and c indicate the status of 19 cubs from 2008–2020. The percentage of cubs born naturally is 63%, while the percentage of stillbirths is 37%. In male cubs, the stillbirth rate was 26%, and in female cubs. The proportion of normally born cubs was higher in females, accounting for 42% and 21% of male cubs, respectively.

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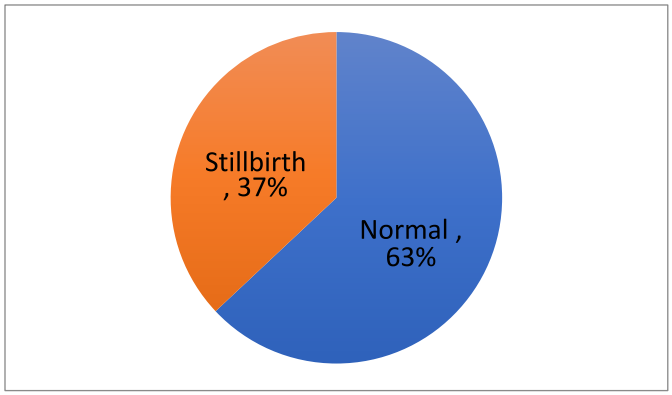
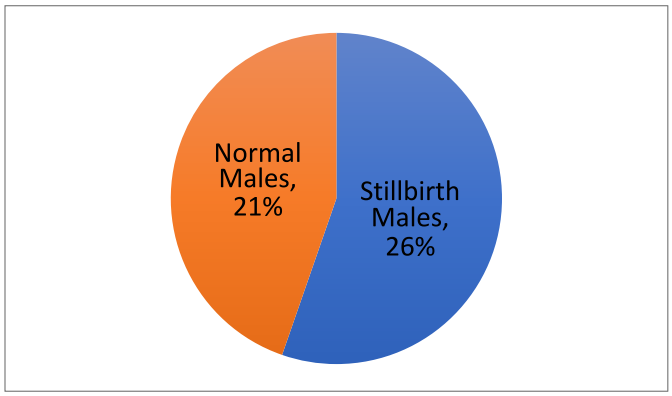
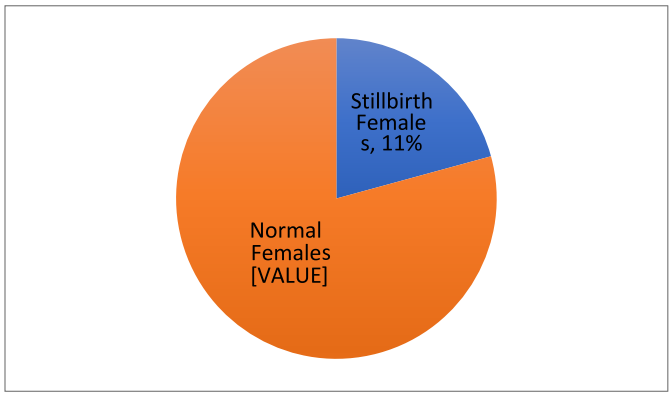


14a: Total Percentage of Normal and Stillbirth



14b: Total Percentage of Normal and Stillbirth in Male Cubs

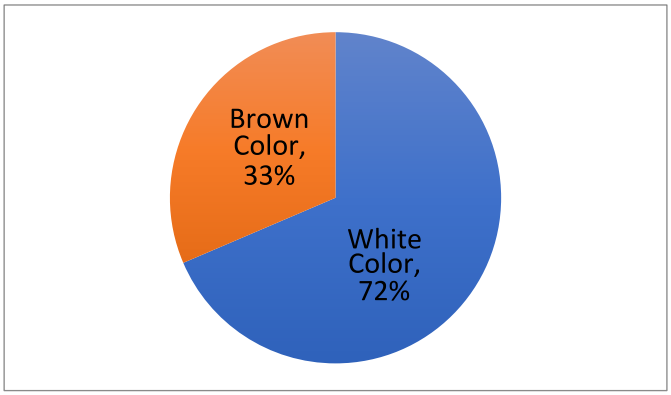


14c: Total Percentage of Normal and Stillbirth in Female Cubs

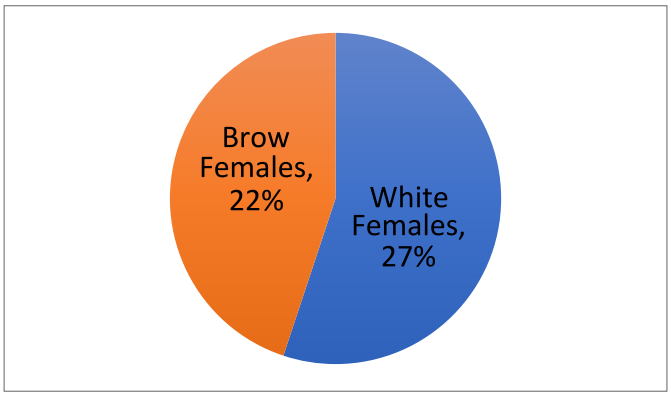
Figure 14: The Birth Status of Cubs 2008-2020

Gender and Color Ratio of Cubs 2008-2020

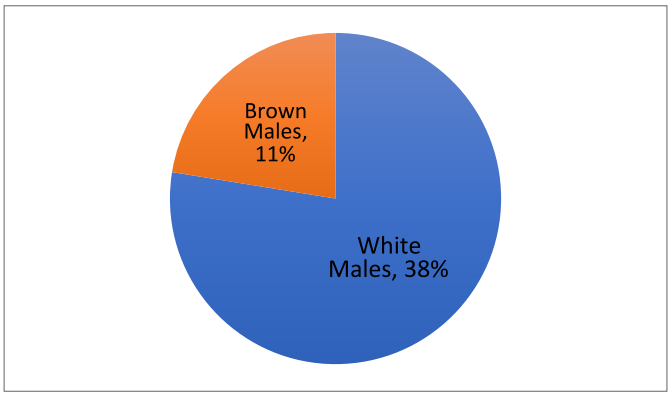
Figures a, b, and c represents the gender and color ratios of 181 cubs from 008–2020. The percentage of brown cubs is 33%, while the percentage of white cubs is 72%. The white male cubs were 38%, while the female cubs were 27%. The brown-colored cubs were more common in females (22% and 11% in male cubs, respectively).



15a: Total Percentage of White and Brown Color in all Cubs



15b: Total Percentage of White and Brown Color in all Female Cubs



15c: Total Percentage of White and Brown Color in all Male Cubs

Figure 15: The gender and color ratio of cubs born between 2008 and 2020

DISCUSSION

Due to low genetic variation, cases of inbreeding depression were common in the Lahore Zoo. In 2008, five cubs were born in the zoo, of which three were stillbirths and two were born normally and died on the same day. Rozi and Jumbo were the parents of these cubs (the Bush and Leo progeny). In 2009, following the inbreeding of Rozi and Jumbo, four cubs were born healthy and again died on the same day. In the year 2010, mother Rozi was again crossed with Sam. At that time, the progeny was 4, and these were 2 stillbirths and 2 live births. In 2010, the same couple again

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gave birth to three stillborn babies [13]. The reason for the deaths of cubs was the abandonment of their mothers. In nature if a new born cub is not healthy the mother will not participate in its rearing and may avoid it. In present reported cases the cubs that were survived were reared on formula milk. These cubs were already suffering from inbreeding and the lack of mother milk made them susceptible to diseases and weakness. In such cases maximizing of genetic diversity is recommended [12-14] because increased in transfers and exchange of animals decrease the inbreeding rates [15]. According to, inbreeding depression can have a variety of detrimental effects on captive zoo populations, mostly by lowering the likelihood that inbred animals will reproduce and survive [13]. The most significant conclusion of this study was that two of the most frequently detected signs of inbreeding depression were increased litter size with deformed cubs and declining longevity. Other characteristics that have been linked to inbreeding include albinism, smaller skeletons, and lower birth weights. Decreased fitness was a sign of inbreeding depression, although it might also be caused by other things like bad management or husbandry techniques. Many of these findings were present in new born cubs of Lahore Zoo tigers. Zoo management took extensive care of the female white cub zone from April to September 2010, but the results were not fruitful. When the mother of Zona stopped feeding her, the dedicated staff of the zoo took her to save the environment of the zoo hospital and started hand-feeding. She gave a good response to these steps, even though 14 and 15 noted a comparable pattern. Even though inbred tigers would not make it through a hostile environment, they'd be fine in a hospitable one. Although the inbreeding coefficient has a negative effect on mortality rate, it has a positive effect on litter size. One possible explanation is that while environmental factors also have a role in determining an animal's lifespan, the additive effect of genes controls litter size [15, 16]. The findings of current study were also similar to that that the litter size from 2008-2020 were up to 5, 4 and 3 but the stillbirth and mortality rate was also in all breeding high with less survival rate that is 63%. Among the 19 births of all cubs from 2008–2020, only 4 lived up to 3 months, and one lived up to 6 months. Many of tiger cubs died on the on the same day of their birth and other were stillborn. The suggested that the overall longevity of the tiger population was also found to be significantly reduced by an increase in inbreeding [17]. In male cubs, the high rate of death was recorded at 26%, and they were 38% white in color. The female cubs were 27 % white, but they born 42% normal in comparison with males male their normal birth ratio was 21%. it is clear from here that white males were survive less , it is important to limit breed of white tigers in captivity so that the problem of inbreeding can be mitigated [18]. Inbreeding negatively impacts the survival of tigers, potentially leading to extinction. Detection of causal mutations for disease is rare for wild animals, thus having the identities of the candidate loci for further verification is very helpful [19]. The loss of genetic diversity and the accumulation of mutations caused by inbreeding within tiger groups warrant more investigation into the possibility of future mutations in the tiger population. Additional research in the possibility of genetic alterations in inbred species, such as tigers, is necessary. Zoos, private breeders, and conservationists should be held responsible for animal inbreeding by organizations and the government if investigations show that the mutations were getting worse with each generation. Inbreeding can result in a higher frequency of genetic abnormalities, less immunity, and lower fertility, compromising the tigers' general health and survival. This is especially troublesome in captive breeding programs, when genetic diversity is already restricted due to tiny population sizes [20, 21]. There has to be a change in the zoo's collection plan. To keep things simple, careful breeding plans should be drawn up. To rule out a recessive gene, genetic mapping is necessary. It is recommended to incorporate a new blood line. We should use artificial insemination. It is recommended to preserve healthy male gametes for potential future usage. More studies should be carried out on all animals kept in captivity.

CONCLUSIONS

All of the region's living things were considered part of the biodiversity, so whether they were in the wild or in captivity, the loss will be just as severe. The inbreeding of tigers has a negative impact on their survivability, which could ultimately cause their extinction. Tiger conservation efforts should prioritize planned breeding above mating between relatives. It was appropriate for the studbook to be kept in a zoo. Tigers were more likely to contract inbreeding and other diseases due to their white coats. Therefore, it's important to resist the urge to breed more white tigers. Captive animals that would have perished in the wild were able to survive inbreeding, thanks to improved management and care. The conservation and care efforts of the animals at the Lahore Zoo have made it famous. Efforts to enhance the lives of confined wildlife have consistently been prioritized by its management. The zoo attempted to solve the inbreeding problem in 2018 by importing fresh tigers, but unfortunately, it was

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unsuccessful. The management should ensure that the new tiger cubs were paired with a complete genetic record so that they can be properly bred in the future.

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Authors Contribution

Conceptualization: BNK, SB
Methodology: BNK, A, SB, MRK
Formal analysis: BNK, ZM, SB
Writing, review and editing: MA, NH, AM, MAH, RZ, A, MZ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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