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Comparative genetic analysis of reproductive parameters between captive white tigers and normal tigers

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Abstract
The genetic analysis of reproductive parameters of the Nandankanan Zoological Park (NZP) tigresses (n=342) were investigated from pedigree data from 1960 till June, 2016. The mean (±SD) of inbreeding coefficient, birth weight, age at first cubing, age at first mating, number of cubs born in lifetime, number of cubs live up to adulthood, average litter size, total number of white cubs born, and sex ratio (Male : Female) showed highly significant (P<0.01) difference between the normal and white tigers. By PCA, it was illustrated that total cumulative variation of 78.10 % develop by four factors i.e., PC1, PC2, PC3 and PC4 eigen value of 4.013, 2.52, 2.19 and 1.23, respectively. Most of the reproductive parameters were found to be high to moderately heritable, that means the tigress were more genetically active to produce new progeny with most desirable genes for next generation.

Keywords: Breeding value, pedigree, principal component, traits, tiger

1. Introduction

Tigers are consider as endanger species according to International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species [1] and are also protect under Schedule I of The Indian Wildlife (Protection) Act (IWPA), 1972 [2]. The tiger population suffered an estimate decline of more than 50% in the last three decades, with a total effective breeding population size closure to 2,500 and with less than 250 mature breeding individuals [3]. It has been reported that, in the late 1990s, the total tigers survived in the wild had been significantly reduced to 5,000-7,000 [4] and fewer than 1850 tigers were present in South Asia [5, 6]. In India, tiger estimation conducted by government-run Wildlife Institute of India (WII) found to be 1,945-2,491 left in the wild [7]. Tigers now occupy only 7.1% of their historical range [8]. Currently, the total number of breedable wild tiger are consists of fewer than 100 those are mostly restricted to isolated patches of suitable protected habitats in India [9, 10]. So, ex-situ and in-situ management of exiting tiger population through zoological programmes is most important to protect the species from extinction. This practice relies on human intervention through better captive management strategies along with proper selection of heritable parent to pass the desirable gene to next generation through better breeding strategy [11].

It is inevitable to avoid occurence of inbreeding in a small fragmented populations [12]. Ultimately, the small tiger population will face the risk of extinction through the expression of inbreeding depression [12, 13, 14]. So, to counteract the inbreeding depression and to sustain from extinct, genetic variability among the tiger population is an important criterion [15]. Moreover, it has been reported that with decrease in tiger population, the birth of more white tigers occurred due to mating between close relatives with less genetic variability [16]. Simultaneously, lot of deformities and deficiencies begin to surface very soon in white tiger population like higher cub mortality and albinism due to inbreeding depression [16; 17]. Initially researchers believe that white tigers are albino, but actually they are leucistic [17]. Albinism is characterised by the absence of pigment in the skin, hair, and eyes due to an enzyme defect involved in melanin production [18]. Actually, many white tigers in captivity are inbred due to carriage of autosomal recessive trait and consequently suffer from some health issues, leading to the controversial speculation that the white tiger mutation is perhaps a genetic defect [19]. The reproductive fitness traits of the tiger weather normal or white are most adversely affected due to inbreeding such as number of young surviving up to the age of weaning, age at first parturition, age at mating, litter size, gestation period, sex ratio, inter-parturition period, etc. [20]. However, some metric traits such as birth weight, disease condition, life span, etc. are indirectly associated with fitness and affected by increased in the level of inbreeding [21]. Usually, animal populations held in captivity are usually smaller than wild populations, which mean that the chances of occurrence of inbreeding might be higher in captivity. Therefore, inbreeding depression can leads to more problematic in terms of reproduction, for producing the next generation cubs by choosing the best individual with highly heritable reproductive traits as parents [22]. So, it is utmost important to have a good understanding of reproductive parameters in zoo populations, in order to maintain the survival of species [23]. Therefore, the present study aimed to have a genetic analysis on maternal reproductive traits of both white tiger and normal tiger in response to genetic and phenotypic variance. Also, a comparative study was carried out in both the colour type tigers to determine the relation between each of the fitness (reproductive) traits to pass on to next generation by determining the heritability.

2. Material and Methods

2.1 Resource population

Present study was performed on normal and white tigers by collecting the pedigree information from tiger studbook of NZP, Bhubaneswar, Odisha, India. The zoological park is situated at a longitude of 85˚ 48' 09" to 85˚ 48' 13" E and latitude of 20˚ 23' 08" to 20˚ 24' 10" N at an altitude of 45 meters above the sea level. The park region belongs to tropical climatic zone with average rainfall ranging from 1200 mm to 1400 mm during monsoon and rainy season. During summer, the average temperature rises to 40˚C which drops by 10˚C in autumn and falls to an average of 15˚C during winter season. From the day of establishment of zoological park, since 1960 till June, 2016, 342 tigers have lived. Among these 342 tigers, 161 and 178 tigers were male and female respectively while gender of 3 tigers were not detected due to early death with undeveloped genital organs. In terms of body coat colour, 136 and 206 tigers were having white and normal body coat colour till the time study was carried out. Out of these 178 females, only 27 females with 9 white colour and 18 normal colours have been used for breeding purpose as parent to pass the gene to the next generation. According to the zoo guideline, animals were kept with well-organized managemental practices.

2.2 Data collection

The information on different reproductive parameters along with date of birth, date of death, birth weight were collected and calculated from the tiger studbook data provided by the zoo authority for the period of 1964 to 2016. The path of pedigree for each tiger was determined and inbreeding coefficient (F) of each tiger was calculated [24]. The reproductive parameters i.e. birth weight, age at first cubing, age at first mating, parity, total number of cubs born in life time, number of cubs live up to adulthood, age at death, litter size, number of cubs born, number of normal colour cubs born, number of white cubs born, gestation period, average inter-cubing period and sex ratio were measured for each mother tigress.

2.3 Statistical analysis

After compilation of all reproductive parameters of 27 female tigresses, the descriptive statistical analysis i.e., means ± SD (Standard deviation), SEM (Standard error of mean), Range, 95% CL (Confidence level) of mean was done separately for white tiger, normal tiger and entire population. The significance difference in the mean value was detected by independent t-test for normally distributed data. Non-parametric Mann Whiteny U-test analysis was done for the variables that violated the normality. The normality test was done by Kolmogorov-Smirnov and Shapiro-Wilk test. The Breusch-Pagan and Koenker test were carried out to observe the heteroscedaticity of the data for further multivariate analysis of reproductive parameters. Heritability estimates for different traits were obtained by paternal half-sib correlation method. The genetic and phenotypic correlations with standard errors were calculated from variance-covariance analysis [25]. Two types of multivariate analysis: principal component analysis (PCA) and multiple regression were conducted. Statistical power of multiple regression analysis was compromised when the outcome ratio of sample size to independent variables was below five. The Bartlett's sphericity test was conducted to test the significance of correlation among the parameters and then subjected to PCA on the whole reproductive variables to reduce them to a smaller number of principal components that could explain most variables in the original data set [26]. Multiple regression analysis was used to obtain the partial correlation coefficients with statistical significance value between reproductive parameters. Before multivariate analysis, the non-parametric data were converted to normality form by log transformation. All processing of data was done with the software package of Microsoft Excel 2010 for data storage and SPSS version 21 for statistical analysis. The p-values $p<0.05$ and $p<0.01$ with an alpha level of 95% were assumed as statistically significant (*) and highly significant (**), respectively.

3. Results

Basic descriptive statistical analysis on different reproductive parameters of entire population was determined (Table 1). The normality test illustrated that the inbreeding coefficient, parity, number of cubs live up to adulthood and sex ratio (Male: Female) were significantly violating the normality principle when calculation was done based on colour. These data were transformed by log transformation for further univariate and multivariate statistical analysis. By heteroscedaticity test, it was observed that all the variables were not significant ($P<0.05$) that means all the parameters were linearly distributed. The homoscedaticity normality distributed variables and log transformed non-parametric variables were subjected to PCA and multiple regression analysis.

3.1 Descriptive statistical analysis of reproductive traits

Mean value of each reproductive parameters along with the fitness traits based on colour were compared to evaluate the significant difference. After comparing the mean of these reproductive traits, it was found that inbreeding coefficient, birth weight (in kg), age at first cubing (in days), age at first mating (in days), number of cubs born in lifetime, number of cubs live upto adulthood, average litter size, total number of white cubs born, and sex ratio (Male: Female) were higher in white population as compared to normal tigers whereas parity, age at death (In days), total number of normal cubs born and average inter-cubing period (In days) were higher in the normal colour tiger as compared to white tigers. Mean (± S.D) of inbreeding coefficient, number of cubs live upto adulthood and of sex ratio (Male: Female) was found to be significantly ($P<0.05$) higher in white colour as compared to normal colour (Table 1). Mean (± S.D) of birth weight (in kg), total number of white cubs born was found to be significantly ($P<0.01$) higher in white colour as compared to normal colour (Table 1). Mean (± S.D) of total number of normal cubs born was found to be significantly ($P<0.05$) higher in normal colour as compared to white colour (Table 1). However, other reproductive parameters i.e. age at first cubing (in days), age at first mating (in days), parity, number of cubs born in lifetime, age at death (in days), average litter size, gestation period (in days) and average inter-cubing period (in days) did not show any significant difference (Table 1).

3.2. Genetic and phenotypic correlation between reproductive traits

The genetic and phenotypic correlation between various reproductive traits was calculated (Table 2). In the entire tiger population, the gestation period was found to be non-significantly positively correlated with litter size, inter-cubing period, birth weight and age at death genetically whereas negatively correlated with parity, number of cubs born in lifetime, number of cubs live upto adulthood, age at first cubing and age at first mating (Table 2). The genetic correlation coefficient of parity with number of cubs born in lifetime (0.944 ± 0.370) and age at first cubing (0.886 ± 0.239) were found to be positively significantly ($P<0.05$) correlated while inter-cubing period (0.896 ± 0.212) was negatively significantly ($P<0.05$) correlated (Table 2). Furthermore, parity was found to be genetically non-significantly positively correlated with age at first mating and negatively correlated with litter size, number of cubs live up to adulthood, birth weight and age at death (Table 2). Similarly, positive value of genetic correlation coefficient was observed between number of cubs born in lifetime with age at first cubing (0.993 ± 0.044) and age at first mating (0.953 ± 0.4347) with statistical significant value ($P<0.05$) whereas negative significant ($P<0.05$) value was found with inter-cubing period (0.993 ± 0.045) (Table 2). However, the genetic correlation of number of cubs born in lifetime with birth weight and negatively correlated with litter size, number of cubs live up to adulthood and age at death was found to be positive without any significantly value (Table 2). Nevertheless, litter size was genetically evaluated to be negatively correlated with birth weight (0.867 ± 0.208) with statistical significant value ($P<0.05$). Moreover, the genetic correlation between age at first cubing and inter-cubing period (0.895 ± 0.214) was found to be positively correlated. However, litter size, age at first mating, number of cubs live up to adulthood, age at death and inter-cubing period were assessed to have no significant genetic correlation among them (Table 2).

3.3 Principal component analysis

According to the result of Bartlett's sphericity test, the null hypothesis was rejected when the correlation matrix of all reproductive variables was an identity matrix and all correlations were zero ($P<0.01$) [26]. The principal components analysis resulted in four factors, which accounted for cumulative total variation of 78.10 % (Table 3). PC1 was positively correlated with number of cubs born in the lifetime, parity and number of cubs live up to adulthood. The PC2 was positively correlated with age at first cubing (in days) and age at first mating (in days). The PC3 was positively correlated with birth weight (in kg) and total number of white cubs born. The PC4 was positively correlated with average litter size. The Eigen value of PC1, PC2, PC3 and PC4 was found to be 4.013, 2.52, 2.19 and 1.23, respectively (Table 3). The variable showing a factor loading of more than 0.785 was taken into consideration for that particular principal component. The factor loading developed by number of cubs born in the lifetime, parity and number of cubs live up to adulthood in PC1 were 0.931, 0.891 and 0.822. They were most closely correlated to each other to produce 28.7% of total variation in PC1. Similarly, age at first cubing (in days) and age at first mating (in days) developed a factor loading of about 0.912 and 0.902 to produce 18% of total variation in PC2. Birth weight (in kg) and the total number of white cubs born developed a loading factor of 0.879 and 0.792 to produce 15.6% of total variation in PC3. Average litter size developed a loading factor of 0.781 to produce 8.8% of total variation in PC4 (Table 3).

3.4 Heritability of reproductive traits

Among the reproductive traits, litter size (0.703 ± 0.094), inter-cubing period (0.499 ± 0.074), number of normal cubs born (0.471 ± 0.109) and age at first cubing (0.432 ± 0.068) were estimated to have high heritability values (Table 4). While the traits like birth weight (0.316 ± 0.033), number of white cubs born (0.371 ± 0.064), age at death (0.282 ± 0.043), age at first mating (0.245 ± 0.076) and gestation period (0.226 ± 0.096) were found to be moderately heritable whereas number of cub's live up to weaning (0.042 ± 0.085) and number of cubs born in lifetime (0.054 ± 0.080) were estimated to have low heritability values (Table 4).

3.5 Linear regression analysis of reproductive traits

Potential effects of colour on the different reproductive parameters were also investigated; simple bivariate plots were constructed and the fit for a regression model was tested for each parameter (Table 5). The correlation between age at first cubing (In days) and number of cubs live up to adulthood was calculated (Fig. 1) which showed statistically negative significant ($P<0.01$) relationship having moderate regression coefficient value (R^2=0.177). Correlation of parity with number of cubs born in the lifetime, number of cubs live up to adulthood, total number of normal cubs born and gestation period (in days) were calculated (Fig. 2 and Fig. 3) which showed positive significant ($P<0.01$) relationship. All these parameters showed high regression coefficient values (R^2=0.844, R^2=0.378, R^2=0.453 and R^2=0.648, respectively). Similarly, correlation between the number of cubs live up to adulthood and total number of white cubs born was found to be statistically significant ($P<0.01$) with moderate value (R^2=0.278) of regression coefficient (Fig. 4). The correlation between the number of cubs born in lifetime with number of cubs live up to adulthood, total number of white cubs born and total number of normal cubs born were found to be positively significant ($P<0.01$). The corresponding regression coefficient values were calculated (R^2=0.648, R^2=0.270 and R^2=0.324, respectively) (Fig. 5 and Fig. 6). Furthermore, correlation between birth weight (in kg) and total number of white cubs born were evaluated (Fig. 7). The value of regression coefficient (R^2=0.377) depicted highly significant ($P<0.01$) correlation. Relationship between birth weight (in kg) and total number of normal cubs born revealed a negative significant ($P<0.05$) correlation (Fig. 8) with moderate regression coefficient (R^2=0.156). The inbreeding coefficient and the number of cubs live up to adulthood was found to be negatively correlated (Fig. 9) with statistical significant ($P<0.05$) value and moderate regression coefficient (R^2=0.145). A similar correlation between inbreeding coefficient and average litter size was observed (Fig. 10) which were found to be positively significantly ($P<0.05$) related with moderate regression coefficient (R^2=0.259). The correlation between total number of white cubs born and total number of normal cubs born also showed positive significant ($P<0.01$) relationship with moderate regression coefficient value (R^2=0.165). On contrast to the above, correlation between average litter size, gestation period (in days), sex ratio (Male: Female) and the total number of white cubs born did not show any significant relationship with each other.

4. Discussion

The challenge faced by the researcher while studying the pedigree data were the variation arises due to past data availability, organization of data, evaluation of data and statistical analysis that might affect the results. The different factors both extrinsic and intrinsic, can affect the reproductive variables should be considered while establishing and using reference intervals. Extrinsic factors include factors that may stress the animal, managemental practices and habitat whereas intrinsic factors are associated with host characteristics [27, 28]. Calculation of reproductive values of 27 captive tigresses out of 178 tigresses that passed the progeny to next generation was determined as standardized value (Table 1).

4.1 Genetic factor responsible for development of white tiger

All mean reproductive parameters of the entire population based on colour were calculated (Table 1). The white tigers were found to have significantly higher inbreeding coefficient than that of normal female tigers. It could be explained by the fact that an increase in inbreeding leads to the recessive homozygous condition where white coat colour is recessive to normal coat colour in tigers. So, it was predicted that inbreeding may be responsible for the development of white coat colour which was in agreement with the previous findings [20, 16]. Birth weight, average number of cubs live up to adulthood and sex ratio (Male: Female) were found to be significantly higher in white female tigers as compared to normal tigress. The white tigress might be genetically adapted to tropical habitat condition of zoological park than that of a normal colour tigress, as a result carry the genes that allow them to survive and thrive more conveniently by producing the male individuals. But our findings contradict to the previous study at Ranthambhore Tiger Reserve (RTR) from the period 2006-2014 where the overall average sex ratio was female dominant. During the initial year (2006-07) of studies adult sex ratio (Male: Female) in RTR was female biased (0.38) but in the subsequent years (2012-14) it became marginally female biased (0.91). However, the mean adult sex ratio (Male: Female) ratio was revealed to be 0.76 during the entire study period [29]. Moreover, the number of cubs produced by the tigress was found to be more in their respective colour groups which can be explained as the gene responsible for the development of colour was transmitted from parent to the offspring sporadically in the respective colour group.

4.2. Standardized value of reproductive traits in tiger

The average age of first cubing in tiger was found to be 5.84 ± 0.43 years. The minimum and maximum age at which the tigress was able to produce the first cubs were 3.87 years and 11.65 years, respectively (Table 1). Earlier it was reported that tigress mostly mated between 2 to 16 years of age in the captivity [30]. Almost satisfying to the previous findings, in NZP, the tigresses were breed from 4 years to 15 years. It had been established that female tigers in their lifetime were reproductively active until 14 years of age [31, 32; 33]. In Nepal, the maximum known-aged tigresses which produce cubs were found to be 15.5 years of age [34]. The average parity of NZP tigers was found to be 4.15 ± 0.42. The number of cubs born in each parity ranges from 1-9 cubs in different tigresses for their entire lifetime (Table 1). Average total number of cubs born in the life of a mated tigress was found to be 12 ± 1 (Table 1), which showed that the tigers were more adapted reproductively to the tropical climate of Odisha. Moreover, white tigresses produced more offspring than that of the normal colour female tigers. So white tigers were more genetically active to reproduce and well-adjusted to the environmental condition. The estrous period of NZP was determined to be 5-7 days by manual observation with an average gestation period to be 102.6 ± 0.75 days with a range from 92 to 107 days (Table 1). Sunquist [30] reported that the female tigers had 6 days of esterous cycle and 107 days of gestation period, while Mazak [35] studied 3-6 days of esterous cycle and 104 days of gestation period in the captive tigers. Further, he elaborated that the gestation period in tigress's ranges from 96 to 111 days. In another study by Sunquist [30] observed that the old tigress of thirty month female also biologically active for esterous cycle. Moreover, the NZP tigress had the mean inter-cubing period of 19.23 ± 1.87 months with minimum and maximum age differences between two consecutive births in the same group of animals ranged between 6.43 to 41 months (Table 1). The average inter-cubing period of NZP tigresses was found to be less than Chitwan National Park, Nepal tigresses (21.6 months, n=7) [36], Panna Tiger Reserve tigresses (21.6 months, n=14) [37] and also to the Amur female tigers (21.6 months) [38]. However, it has been reported that the mean inter-cubing interval of RTR tigresses (33.4 months, n=15) was much higher than in any other reported studies on Bengal tiger [39]. The longer average inter-cubing period in RTR might be due to the influence of harsh climatic conditions during summer and the low annual precipitation [39]. In addition, the average litter size of NZP female tigers in captivity was found to be 2.87 ± 0.12 which ranges from 1.50-4.00. These findings almost nearer to the previously reported mean litter size (2.9±0.2) of Pench Tiger Reserve (PTR), Madhya Pradesh tigresses [39]. The phenomenon can be explained by the fact that as the NZP tigresses reproductive traits including average litter size is genetically active analyzed through its heritability (0.703±.094) concept. Moreover, the tropical climatic condition of NZP is more suitable for these Bengal tigers survivability, as environmental effect plays an important role for phenotypic variation. And, lot of phenotypic variation in the reproductive traits were seen in the NZP tigress. As, both the regions (NZP, Odisha and PTR, Madhya Pradesh) are having almost same climatic condition, so, the average litter size of these tigresses were found to be almost same. But the mean litter size of NZP was found to be much higher than that of Ranthambhore Tiger Reserve (RTR), Rajasthan (2.3±0.12) [39], that may be due to adverse and hurtful climatic condition of Rajasthan as compared to Odisha. In wild, litter size of tiger ranged from one to seven cubs with an estrous cycle of five to seven days [30].

4.3 Longevity of tiger population

In NZP, it was calculated that the female tigers survive in the zoo for 15.01 ± 0.83 years, however the normal colour female tigers survived little bit higher (15.91 ± 1.09 years) as compared to the white tigresses (13.87 ± 1.53 years) (Table 1). Moreover, the maximum and minimum age to which the tigresses survived were 6.59 years and 22.84 years, respectively (Table 1). It justified that the longer survivability of the tigers in the captive condition as compared to natural habitat due to better managemental practices adopted by the authority towards its longevity. In the present study the average age at which tigress were able to reproduce maximum litters with healthy cubs that survived upto weaning was found to be 7.04 years which is almost one year ahead in age (6.2 years) as compared to previous reported study [30]. This difference raised may be because of better managemental practice to produce more healthy cubs in the lifetime of a female tiger. But still the percentage of cubs live up to adulthood was found to be 55.12 % with mortality of cubs before one year was nearly 41 %. This mortality percentage could be explained due to tropical environment which is considerably little stressful to young borned cubs due to extreme heat, humidity and parasites. Still the survivability percentage of new borned tiger cubs was higher in tropical climate as compared to other habitat due to tropically adapted genotype were genetically more resistant to heat and parasites.

4.4 Genetic analysis of correlated reproductive traits in tiger

Genetically, the importance of quantitative reproductive traits association with fitness represents the larger fraction of their total genetic variance in terms of dominance and epistatic variance as compared to the traits that were less closely associated with fitness [24, 40]. If any two parameters are genetically positively correlated, then the favorable response to the selection by one parameter will be echoed by related one [11, 41]. Similar concept of findings were also discovered in the present study that the parity and the number of cubs born in lifetime were positively correlated which explained that the tigresses were genetically potent enough to produce more healthy cubs with increase in parity. Moreover, the female tigers that were subjected for early cubing developed increase in number of parity in their entire life as both were highly positively correlated. In the similar manner, if genetic correlations are negatively correlated, improvement in one trait due to selection will result in decline the negatively associated traits [11, 41]. Normally, if, the number of parity in the lifetime increases the time interval between the two consecutive cubing usually decreases. In the present study the parity was negatively associated with the inter-cubing period. Satisfying to the above concept that as the inter-cubing period decreases the total number of cubs born in lifetime increases. As well as, it was justified that the selection of tigress with higher litter size would resulted into decrease in cub's birth weight. Also, the present research illustrated that the tigress allowed to mate and parturate at late adult stages of life produced maximum number of offspring in the entire life because, with increase in age the animal became reproductively active and conceive well. There may be certain genes that affect the growth rate and reproductive traits at specific age of an animal's life. These genes were mostly having pleiotropic response on associated reproductive traits as the traits were genetically correlated. Selection of any traits resulted in correlated response of most closely associated trait in that particular direction [42]. The above rationale might be one of the reasons for high positive genetic correlation between age at first cubing and inter-cubing period in female tigers because both were closely associated reproductive traits.

4.5 Influence of heritability on reproductive traits of tigers

In the NZP tigreses, litter size, inter-cubing period, the number of normal cubs born and age at first cubing were found to have higher heritability values (Table 4). This increase in the heritability of a trait is thought to occur through the conversion of non-additive genetic variation to additive genetic variation [43], perhaps as an evolved response to fluctuations in population size through time [44]. When heritability was high, phenotypic values generally revealed more about breeding value of that particular parameter. Thereby, it would be easier to determine the tigress with higher breeding value could become the best potential parent for the next generation. For moderately heritable traits like birth weight, number of white cubs born, age at death, age at first mating and gestation period (Table 4), the prediction for the accuracy of selection would be negligible which means the rate of genetic change was expected to be slow due to poor breeding value. The relationship between heritability and reproductive traits may be developed due to the conversion of non-additive genetic variance to additive genetic variance through decrease in population size or adaptation to the environmental condition [24]. In this research it was found that the genetic correlation of heritability and reproductive traits were high. So, proper parental choosing would be important for passing these heritable traits to the next generation. In contrast, the traits like number of cub's life up to weaning and number of cubs born in lifetime (Table 4) of low heritability value indicates that proper managemental practice or environmental condition as compared to genetic selection would be important for recovery of these traits. The higher percentage of environmental factors affecting a trait, this phenomenon more likely indicate that the trait would be developed through improved management or genetic techniques like out-breeding.

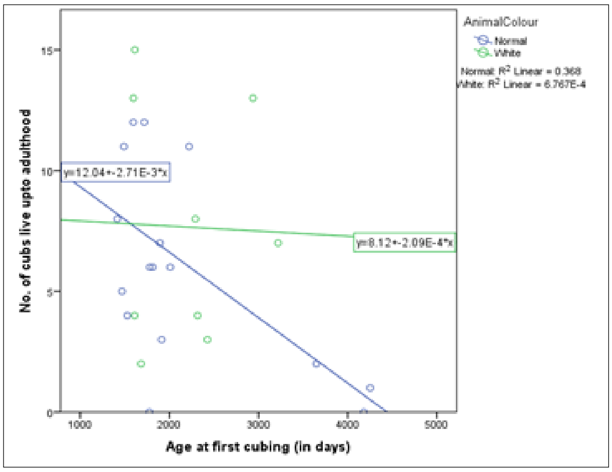

Fig. 1 Relationship between age at first cubing (in days) and number of cubs live upto adulthood

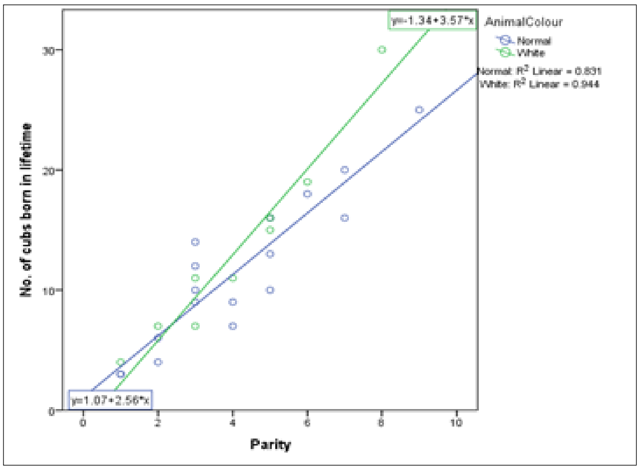

Fig 2: Relationship between parity and number of cubs born in lifetime

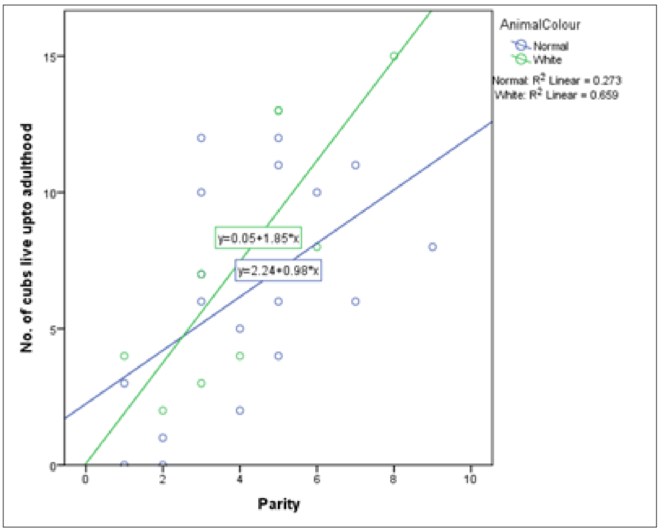

Fig 3: Relationship between parity and number of cubs live up to adulthood

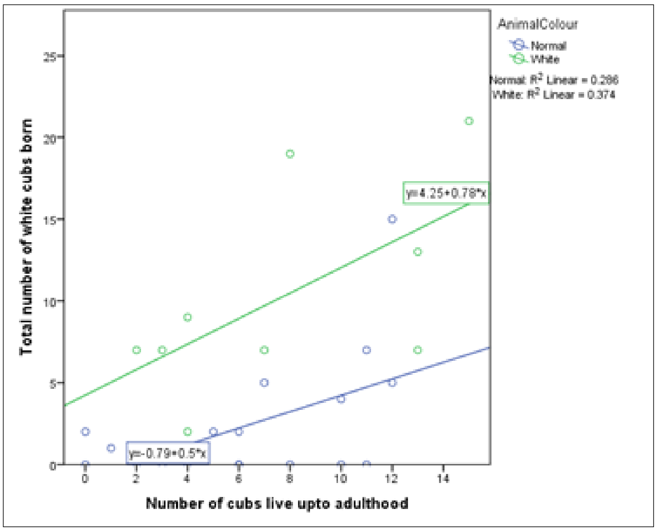

Fig 4: Relationship of number of cubs live up to adulthood and total number of white cubs born based on body colour

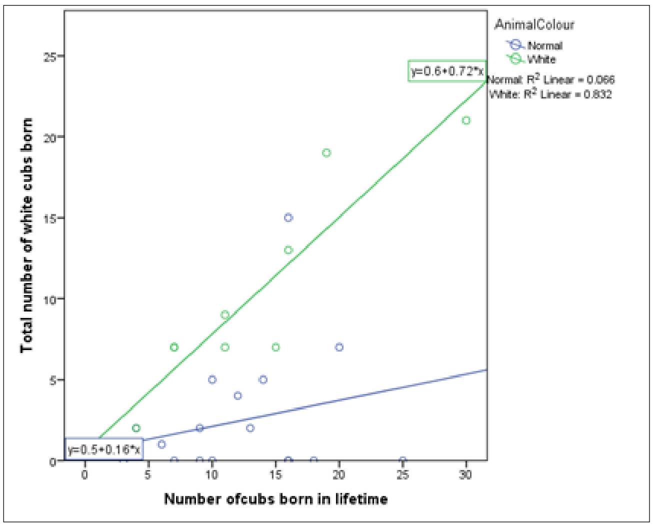

Fig 5: Relationship between number of cubs born in lifetime and total number of white cubs

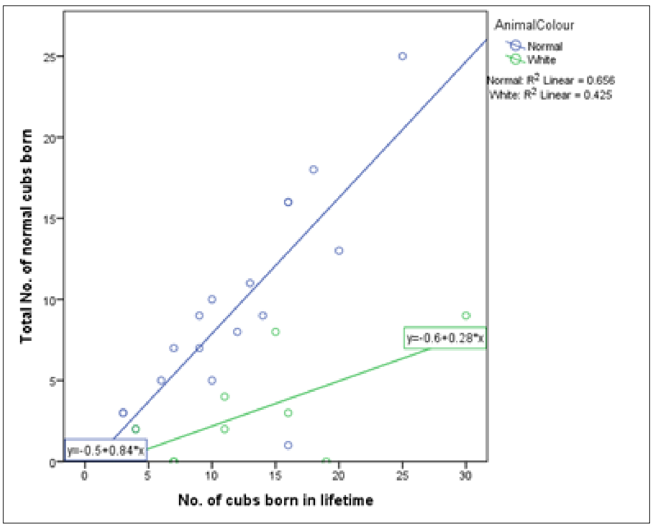

Fig 6: Relationship between number of cubs born in lifetime and total number of normal cubs born

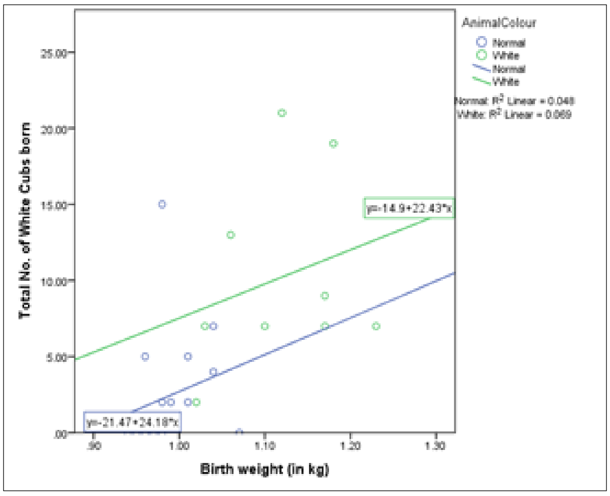

Fig 7: Relationship between birth weight (in kg) and total number of white cubs born

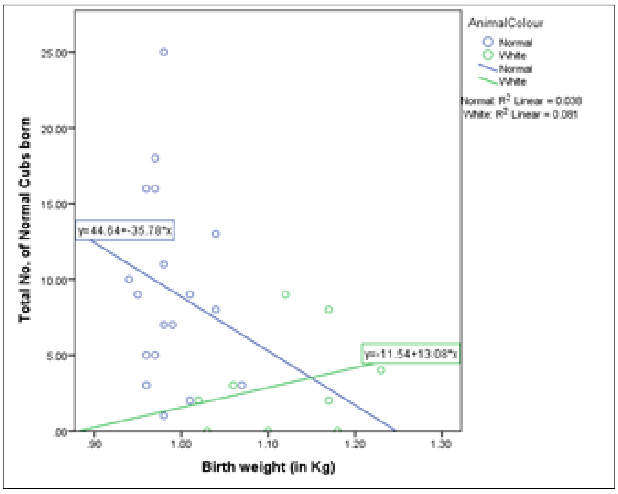

Fig 8: Relationship between birth weight (in kg) and total number of normal cubs born

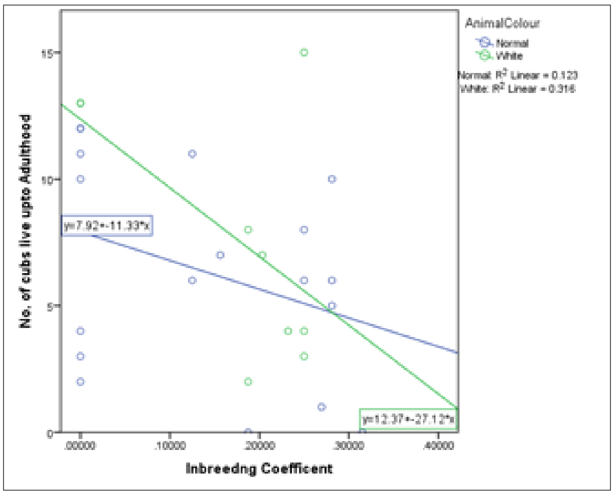

Fig 9: Relationship between number of cubs live up to adulthood and inbreeding coefficient

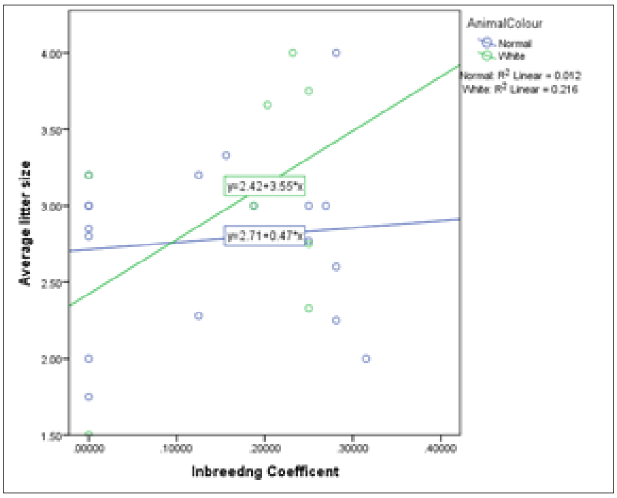

Fig 10: Relationship between inbreeding coefficient and average litter size

Table 1: Comparison of reproductive traits in Normal and White tiger

Reproductive parameters	Normal colour			White colour			P-Value	All Tiger					
	Mean±S.D (N=18)	95% CL of Mean		Mean±S.D (N=9)	95% CL of Mean			Mean±SEM (N=27)	S.D	Range		95% CL of Mean	
		LCL	UCL		LCL	UCL				Minimum	Maximum	LCL	UCL
Inbreeding coefficient	0.148± 0.03	0.079	0.198	0.193±.034	0.100	0.259	[b]0.025*	0.151±.022	0.118	0.000	0.315	0.105	0.205
Birth weight (in kg)	0.988± 0.008	0.972	1.00	1.12±.024	1.08	1.16	[a]0.000**	1.03±.016	0.081	0.94	1.23	1.00	1.06
Age at first cubing (in days)	2117±208	1744	2545	2188±203	1835	2550	0.809	2133±156	814	1416	4253	1850	2450
Age at first mating (in days)	2016±223	1645	2447	2089±204	1734	2452	0.809	2031±158	816	1309	4156	1749	2353
Parity	4.35±.507	3.22	5.17	4.11±.716	2.89	5.56	0.950	4.15±0.42	2.13	1	9	3.37	4.96
Number of cubs born in lifetime	11.2±1.43	9.06	14.4	14.3±2.63	8.78	19.8	0.056	12.3±1.33	6.64	3	30	9.74	14.8
Number of cubs live upto adulthood	6.53±1.00	4.56	8.22	8.67±1.63	4.67	11.9	[b]0.046*	6.78±0.87	4.33	0	15	5.08	8.48
Age at death (in days)	5808±389	4942	6416	5063±562	3973	6113	0.363	5479±302	1649	2405	8338	4859	6040
Average litter size	2.77±.138	2.53	3.04	3.04±.258	2.56	3.51	0.326	2.87±.124	0.634	1.50	4.00	2.61	3.10
Total number of white cubs born	2.53±.948	0.78	4.61	10.2±2.08	6.45	14.1	[a]0.000**	5.00±1.21	5.98	0	21	2.82	7.59
Total number of normal cubs born	9.71±1.53	6.61	12.1	3.11±1.12	1.11	5.44	[a]0.011*	7.26±1.18	6.21	0	25	5.07	9.67
Gestation period (in days)	99.0±.963	97.1	100	98.3±1.29	96	101	0.813	98.6±0.75	3.91	92	107	97.1	100
Sex ratio (Male : Female)	0.993±0.224	0.562	1.36	1.56±.336	.976	2.15	[b]0.031*	1.15±.187	0.982	.000	3.33	0.774	1.53
Average inter-parturition period (in days)	660±73.2	482	773	484±70.4	344	604	0.264	577±56.1	300	193	1230	465	681

*P<0.05, **P<0.01
*SD- Standard deviation
#SEM- Standard error of mean
*CL- Confidence level
*LCL- Lower confidence level
*UCL- Upper confidence level

[a]Parameteric independent t-test to analyse the difference between mean (±S.D) of reproductive traits
[b]Non-parameteric Mann Whiteny U-test to analyse the difference between mean (±S.D) of reproductive traits

Table 2: Genetic variance (Above the diagonal) and phenotypic variance (Below the diagonal) of reproductive traits of tiger

S. No.	Gestation Period	Parity	Number of cubs born in lifetime	Litter Size	Number of cubs live up to adulthood	Age at first cubing	Age at first mating	Inter-cubing Period	Birth weight	Age at death
Gestation Period	-	-0.116 ± 1.807	-0.397 ± 53.656	0.846 ± 1.153	-0.225 ± 5.708	-0.021 ± 1.842	-0.628 ± 1.561	0.875 ± 1.561	0.458 ± 0.585	0.314 ± 1.629
Parity	0.210 ± .747*	-	0.944 ± 0.370*	-0.250 ± 0.963	-0.834 ± 1.197	0.886 ± 0.239*	0.826 ± 0.494	-0.896 ± 0.212*	-0.572 ± 0.611	-0.642 ± 0.643
Number of cubs born in lifetime	0.542 ± 0.242	0.906 ± 7.436	-	-0.748 ± 1.396	-0.062 ± 12.132	0.993 ± 0.044*	0.953 ± 0.4347*	-0.993 ± 0.045*	0.272 ± 2.599	-0.417 ± 2.790
Litter Size	-0.943 ± 0.901	-0.160 ± 0.561*	-0.927 ± 8.584	-	-0.832 ± 1.121	-0.471 ± 0.803	0.766 ± 0.595	0.651 ± 0.574	-0.867 ± 0.208*	-0.427 ± 1.187
Number of cubs lives up to adulthood	-0.007 ± 0.027*	0.563 ± 2.157	0.480 ± 1.731*	-0.423 ±1.477*	-	0.299 ± 3.612	-0.769 ± 1.067	0.186 ± 3.697	0.913 ± 0.532	0.864 ± 0.981
Age at first cubing	-0.053 ± 0.288*	0.822 ± 5.013	-0.264 ± 0.949*	0.019 ± 0.037*	-0.523 ± 1.940	-	-0.833 ± 0.478	0.895 ± 0.214*	-0.113 ± 0.839	0.231 ± 1.0419
Age at first mating	-0.365 ± 1.358*	0.485 ± 1.909	0.166 ± 0.584*	0.955 ± 11.165	-0.545 ± 2.059	0.206 ± 0.762*	-	0.746 ± 0.670	-0.093 ± 1.267	0.175 ± 1.493
Intercubing Period	0.111 ± 0.355*	-0.417 ± 1.454*	0.364 ± 1.239*	0.832 ± 1.113*	0.461 ± 1.559*	0.934 ± 8.292	0.434 ±1.523*	--	-0.712 ± 0.434	-0.846 ± 0.934
Birth weight	0.313 ± 1.143*	0.267 ± 0.960*	0.677 ± 3.156	0.144 ± 0.505*	0.523 ± 2.128	0.245 ± 0.914*	0.193 ±0.712*	-0.387 ± 1.328*	-	0.682 ± 0.479
Age at death	-0.164 ± 0.576*	0.721 ± 3.610	0.314 ± 1.146*	-0.578 ±0.200*	0.212 ± 0.754*	0.307 ± 1.164*	-0.471 ±1.927	-0.562 ± 2.153	0.284 ± 5.245	-

***** P<0.05

Table 3: Principal component analysis of reproductive traits of tiger

Principal Component	Variables		Percent Variation	Cumulative Variation	Eigen value
	Parameter	Factor loading			
PC1	Number of cubs born in lifetime	0.931	28.7	28.7	4.013
	Parity	0.891			
	Number of cubs live upto adulthood	0.822			
PC2	Age at first cubing (in days)	0.912	18.0	46.7	2.52
	Age at first mating (in days)	0.902			
PC3	Birth weight (in kg)	0.879	15.6	62.3	2.19
	Total number of white cubs born	0.792			
PC4	Average litter size	0.781	8.80	71.1	1.23

*Positive and negative signs preceding variables indicate their orientation on PC axis
*Variables with factor loading above 0.785 were considered important factors in each component

Table 4: Heritability of reproductive traits of tiger

S. No.	Reproductive traits	Heritability ± S.E
1	Gestation period	0.226 ± 0.096
2	Birth weight	0.316 ± 0.033
3	Number of cubs live up to weaning	0.042 ± 0.085
4	Number of cubs born in lifetime	0.054 ± 0.080
5	Litter size	0.703 ± 0.094
6	Number of white cubs born	0.371 ± 0.064
7	Number of normal cubs born	0.471 ± 0.109
8	Age at first cubing	0.432 ± 0.068
9	Age at first mating	0.245 ± 0.076
10	Inter-cubing period	0.499 ± 0.074
11	Age at death	0.282 ± 0.043

Table 5: Multiple regression analysis of reproductive traits of tigers

Reproductive traits (y)	Correlation with other reproductive traits (x)	Regression equation	R^2-value	p-value
Age at 1st Cubing (in days)	Parity	0.925 – 0.271x	0.0765	0.172
	Number of cubs born in lifetime	0.878 – 0.350x	0.122	0.076
	Number of cubs live up to adulthood	0.823 – 0.420x	0.177	0.029*
	Age at death (in days)	0.961 + 0.197x	0.039	0.325
	Average litter size	0.867 + 0.365x	0.133	0.061
	Total number of white cubs born	0.909 - 0.301x	0.091	0.127
Parity	Number of cubs born in lifetime	0.156 + 0.918x	0.844	0.000***
	Number of cubs live upto adulthood	0.622 + 0.615x	0.378	0.001***
	Age at death (in days)	0.728 + 0.572x	0.272	0.005**
	Total number of white cubs born	0.897 + 0.321x	0.103	0.103

	Total number of normal cubs born	0.547 + 0.653x	0.453	0.000***
	Gestation period (in days)	0.805 + 0.352x	0.648	0.000***
Number of cubs live up to adulthood	Age at death (in days)	0.874 + 0.355x	0.126	0.069
	Average litter size	0.923 + 0.278x	0.077	0.169
	Total number of white cubs born	0.722 + 0.528x	0.278	0.005**
	Total number of normal cubs born	0.875 + 0.353x	0.125	0.071
	Average inter-parturation period (in days)	0.916 + 0.289x	0.084	0.149
Number of cubs born in lifetime	Number of cubs live up to adulthood	0.805 + 0.352x	0.648	0.000***
	Age at death (in days)	0.807 + 0.439x	0.193	0.022*
	Total number of white cubs born	0.73 + 0.52x	0.270	0.005**
	Total number of normal cubs born	0.676 + 0.569x	0.324	0.002**
Birth weight (in kg)	Age at first cubing (in days)	0.963 + 0.193x	0.037	0.334
	Total number of white cubs born	0.633 + 0.664x	0.377	0.001***
	Total number of normal cubs born	0.844 – 0.394x	0.156	0.042*
Inbreeding coefficient	Parity	0.969 – 0.176x	0.031	0.381
	Number of cubs live up to adulthood	0.855 – 0.381x	0.145	0.05*
	Age at death (in days)	0.979 – 0.175x	0.031	0.383
	Average litter size	0.941 + 0.243x	0.259	0.022*
Total number of white cubs born	Total number of normal cubs born	0.835 + 0.407x	0.165	0.035*
	Gestation period (in days)	0.918 + 0.287x	0.082	0.147
	Sex ratio (Male : Female)	0.924 + 0.277x	0.076	0.163
Age at death (in days)	Total number of normal cubs born	0.796 + 0.451x	0.204	0.018*
	Average inter-parturation period (in days)	0.661 + 0.582x	0.339	0.001***
Average litter size	Total number of white cubs born	0.901 + 0.314x	0.099	0.11

5. Conclusion
In population genetic studies, incorporation of more individuals is most important for better understanding of the heterozygosity concept in a captive-breeding of an endangered species like tigers. To get better insight to reproductive data of tigers in zoos, it is necessary to obtain more detail and accurate data. The *ex-situ* conservation genetics study recommends that genetic analysis should precede and accompany the *ex-situ* conservation projects in order to avoid inbreeding and outbreeding depression. Moreover, a general standard for the presentation of genetic studies should be established, which would allow integration of the data into a global database. Evidences suggesting that white tigers are inbred, that's why controversy arises breeding of these white tigers. Most importantly, these type of population genetic study data will provide the breeders an idea to take necessary decision either choosing genetically active or critical management practice that may affect specific coat colour tigers survivability and individual health. It can also be used for better management of captive breeding programs. In NZP, it was found that animal with high average inbreeding coefficient are more prone to white coloration as compared to the tiger with lower inbreeding coefficient. But, still large number of high inbreed animals were found to have normal coat colour. Among reproductive traits, gestation period, numbers of cubs born in life time, age at death and birth weight were found to be significantly, both genetically and phenotypically correlated with each other. So, proper idea on breeding and selection of parental tigresses to reproduce new healthy genetically fit cubs is most important. So, knowledge on breeding value of parents, heritability, correlations between major reproductive traits and their transmission to the progeny generation are essential criteria for selection. However, in NZP almost all the reproductive parameters were with high or moderately heritable so, breeder should decide those animals with the best breeding values to become parents of the next generation through genetic improvement with ongoing managemental practice and environmental condition according to these respective traits.

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