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Assessment of inbreeding depression in big cats: Testosterone levels and semen analysis

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Studies on Asiatic lions earlier suggested that they were highly inbred and have very low levels of genetic variations. However, subsequent studies indicated higher degree of DNA polymorphism in Asiatic lions and Indian tigers and suggested that the low genetic variability was a characteristic feature of these species and was not the consequence of intensive inbreeding. Therefore, the present study was undertaken to ascertain whether inbreeding depression has set in these species. A total of 16 tigers and 7 lions from three Indian zoos were evaluated for inbreeding depression effects by analysis of semen samples with respect to spermatozoal number, percentage motile spermatozoa, percentage morphologically abnormal spermatozoa, ejaculate volume and fertilizing ability of spermatozoa. Majority of the animals exhibited good spermatozoal number, high percentage of motile spermatozoa and low incidence of abnormal spermatozoa unlike inbred animals, thus implying that inbreeding depression has not yet affected these animals. The high fertilizing ability of the semen samples and the high levels of serum testosterone further support the view that the Asiatic lions and Indian tigers are not completely inbred.

THE Indian tiger (*Panthera tigris tigris*) and the Asiatic lion (*Panthera leo persica*) are today the most critically endangered animals. The decimation of these mega cats to a few thousand tigers and a few hundred lions could be attributed to habitat shrinkage, large-scale indiscriminate hunting for sport and pleasure and due to poaching for their body parts which are in great demand in China, South East Asia, Middle East, Western Europe and USA[1]. Thus there is a need to conserve these mega cats and increase their numbers.

Species survival is critically dependent on reproductive performance which in turn seems to correlate with genetic heterozygosity[2–7]. Wildt *et al.*[7] based on a comparative study of the Asiatic lions of Gir forest and several African lions (*Panthera leo leo*) established a direct correlation between the lack of genetic variability in the Asiatic lion and the high incidence of morphological abnormal spermatozoa and low levels of the male steroid hormone testosterone[8,9] and predicted[7–9] that the Asiatic lion has suffered a population bottleneck followed by inbreeding. However, a recent study[10] on the tigers and lions of India based on randomly amplified polymorphic DNA (RAPD), microsatellite analysis of five repeat loci and multilocus fingerprinting indicated a higher degree of genetic heterozygosity than reported and they concluded that the low genetic variability may be a characteristic feature of these species and not the result of intensive inbreeding. The present study was, therefore, undertaken to evaluate the extent of inbreeding depression in the Indian tigers and the Asiatic lions based on semen characteristics such as spermatozoal number, percentage motile spermatozoa, incidence of abnormal spermatozoa, levels of serum testosterone and fertilizing ability of spermatozoa. Our analyses corrobo-

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rate Singh *et al*'s.[10] observation in that the majority of the animals exhibit very good semen parameters characteristic of proven fertile animals and do not seem to match that of inbred animals under the grip of inbreeding depression as suggested by Wildt *et al.*[7].

Materials and methods

Animals

Semen and blood samples were collected from adult Indian tigers and Asiatic lions maintained in various zoos in India (Table 1). All the animals were reared in cages with an access to a crawl during the day time and a standard diet of beef (8 kg without bones) supplemented with adequate amount of calcium, was provided six days in a week. Water was always available to the animals.

Collection of semen

Tigers and lions were anesthetized prior to electroejaculation using a combination of ketamine HCl (2.22 to 2.66 mg/kg body weight; Troy Labs, New South Wales, Australia) and xylazine (1.11 to 1.33 mg/kg body weight; Troy Labs, New South Wales, Australia) which were loaded into a projectile dart (Dis Inject, Helpro Health Products and Services, Delhi, India) and injected intramuscularly using a blow pipe or a dart pistol (Figure 1 *a*). By this procedure, it took the animals about 20 to 30 min to be completely under the influence of the anaesthesia. Further, to maintain a surgical plane of anaesthesia till blood and semen samples were collected, which normally took about 30 min, an additional injection of ketamine HCl (50 to 100 mg) was given intravenously.

The anesthetized animals were then shifted out from the cages onto an elevated platform and allowed to lie on their side. Prior to electroejaculation, the entire area surrounding the anus, testis and penis was washed with water, dried with tissue paper and smeared with spirit which was allowed to evaporate. The prepuce and the penis were also cleaned to avoid contamination of the ejaculate. Simultaneously a rectal probe (3.7 cm in diameter) was disinfected with Dettol (Reckitt and Colman of India Limited, Calcutta, India), lubricated with white petroleum jelly and gently inserted into the rectum of the animal such that the three longitudinal electrodes of the probe faced the ventral side of the animal against the male accessory sex organs (Figure 1 *b*). The probe was connected to a electrostimulator (Bioelectric Co., Washington, DC, USA) consisting of a voltmeter (0 to 45 V), current meter (0 to 4 amp), a control knob to set the desired volt and an on/off switch

Table 1. Collection of semen from captive tigers and lions from various zoos in India

Zoo	Number of adult males tried	Number of animals which electroejaculated	Animals which electroejaculated (%)
Nehru Zoological Park, Hyderabad			
Tigers	6	5	83
Lions	3	3	100
Sakkarbaug Zoo, Junnagadh			
Lions	2	2	100
Nandankanan Zoological Park, Bhubaneshwar			
Tigers	15	11	73
Lions*	2	2	100

*At the Nandankanan Zoological Park an additional 12 hybrid lions (Asiatic × African) which were vasectomized were also tested by electroejaculation to evaluate whether the animals had been successfully vasectomized.

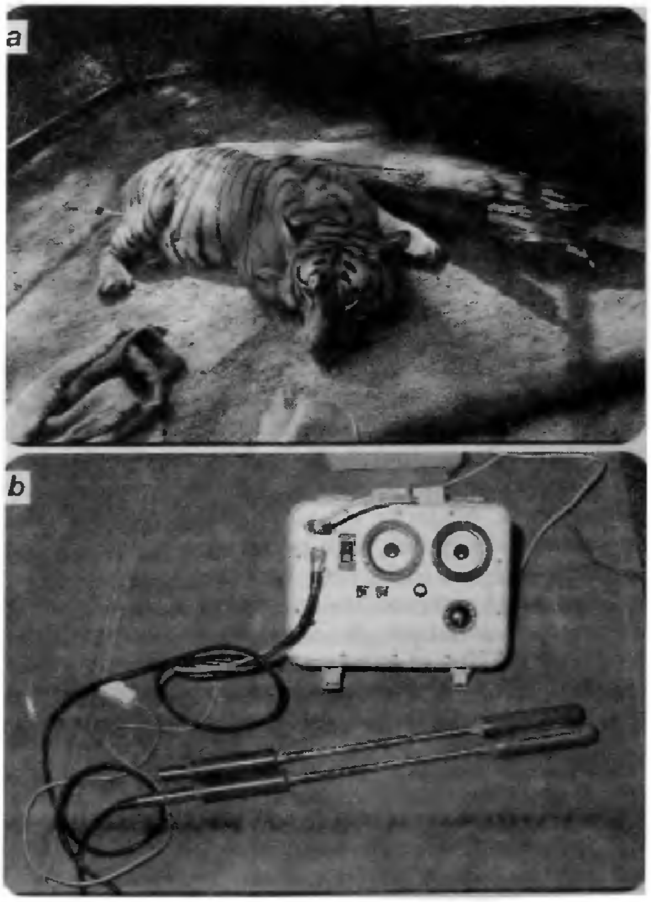

Figure 1. *a*, A tiger which was successfully anesthetized using a blow pipe loaded with a projectile dart (with red feathers) containing ketamine and xylazine; *b*, The electrostimulator and the rectal probes used for electroejaculation of tigers and lions.

to deliver the electric pulses (Figure 1 *b*). In the field the electrostimulator was powered using an UPS (Kirolosker Electricals, Mysore, India). Just prior to stimulating the animal, the penis was extended beyond the prepuce and inserted into a 25 ml sterile specimen container (Figure 2 *a*). The stimulatory regime used for electroejaculation of tigers and lions consisted of a total of 80 stimuli between 2 and 5 V divided into 3 series. In series 1 the animal was stimulated 30 times with 10 stimuli at 2, 3 and 4 V respectively; in series 2 also it was stimulated 30 times with 10 stimuli at 3, 4 and 5 V respectively and in series 3 the animal was stimulated 20 times with 10 stimuli at 4 and 5 V respectively. At times if the animal did not ejaculate even after the third series additional 10 stimuli were given at 5 and 6 V respectively. Each stimulus at the desired voltage was given for 2 s and was followed by a break of 2 s till the next stimulus was given. The animals were rested for 3 to 5 min between each series of stimuli.

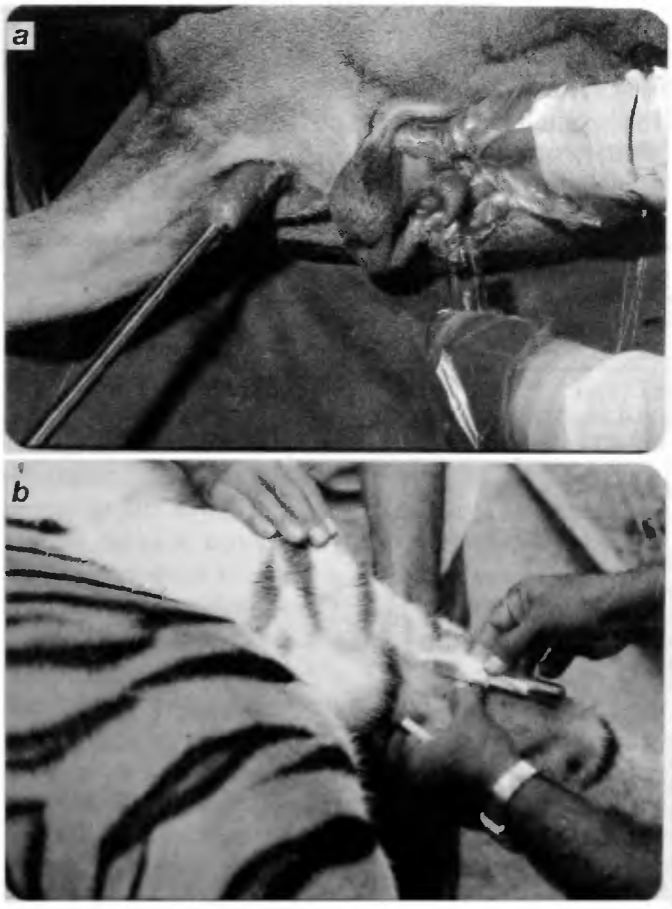

Figure 2. *a*, Positioning of the rectal probe and semen container prior to collection of semen from a lion; *b*, Collection of blood from a tiger using a vacutainer tube.

Determination of semen characteristics

At the end of each series of stimuli, the volume of the ejaculate collected was recorded and an aliquot was immediately evaluated for percentage sperm motility at 400× magnification using a phase contrast microscope. A minimum of three separate fields at 400× were examined to determine the percentage sperm motility. Ultimately the percentage sperm motility reported for an animal was an average of the percentage motility recorded for the ejaculates obtained at the end of each of the three series of stimulations. Ejaculates were pooled for further studies only if they had similar percentage sperm motility. pH of the ejaculates was recorded using pH indicator strips. Sperm concentration per ml of ejaculate was determined using a haemocytometer. For this purpose the semen was diluted in a semen diluent containing 50 g of sodium bicarbonate and 10 ml of 35% formalin per litre of distilled water[11]. Further, an aliquot of the ejaculate was smeared onto 3 clean slides, air dried, fixed in equal parts of 95% ethanol and ether for 5 to 15 min, stained with haemotoxylin papanicolaou[11] and about 500 cells were observed at 400× or 1000× for normal and pleiomorphic spermatozoa. Aliquots were also stained with chlortetracycline (CTC), a fluorescent dye, to ascertain the morphology of the spermatozoa of tigers and lions. For CTC staining semen was washed free of seminal plasma by centrifugation at 800 *g*, the sperm pellet suspended in 500 µM CTC in a buffer containing 20 mM Tris and 130 mM NaCl of pH 7.8 and the suspension was observed under a fluorescent microscope using a 450 to 490 nm excitation filter.

Hamster zona-free oocyte penetration

The hamster zona-free oocyte penetration assay was essentially according to the method described by Yanagimachi[12]. Adult female hamsters on day one of the estrous cycle were injected intraperitoneally with a pregnant mare's serum gonadotropin (40 IU) followed by another intraperitoneal injection of human chronic gonadotropin (40 IU) 48 to 72 h after the first injection. Animals were sacrificed between 16 and 18 h post hCG by cervical dislocation, oviducts removed and transferred to pre-equilibrated buffer TL-HEPES[13] and oocytes were released by puncturing the ampullary region. The oocytes along with the cumulus complex were transferred to a drop of medium containing hyaluronidase (100 µg of hyaluronidas per ml of medium) and gently pipetted to separate the oocytes from the cumulus mass. The oocytes were then washed thrice and incubated separately with trypsin (100 µg per ml of TL-HEPES) till the zona disappeared. The zona-free oocytes were washed thrice and transferred to TALP[14,15]. Meanwhile, spermatozoal

suspensions of the tiger and lion were incubated in TALP-PVA and after 3 h about 100 µl of the sperm suspension (5×10^6 spermatozoa per ml) was used to inseminate 50 µl drop of TALP containing about 10 zona-free oocytes. The drop was covered with liquid paraffin and incubated for 3 h at 37°C in the presence of 5% CO_2. After co-incubation for 3 h, the oocytes were washed repeatedly to get rid off loosely adherent spermatozoa and transferred to a drop of TL-HEPES held between a glass slide and a cover slip supported by drops of soft paraffin at the four corners. The oocytes were then fixed with a drop of 1% glutaraldehyde, dehydrated with alcohol, stained with a few drops of lacmoid or aceto-orcein. Each oocyte was examined under a phase contrast microscope at 500 × to determine the percentage of oocytes which have spermatozoa in their cytoplasm and also the number of hamster spermatozoa inside each oocyte.

Collection of blood and radioimmunoassay of testosterone

Blood was collected from the femoral vein in non-heparanized vacutainer glass tubes (Becton Dickinson, New Jersey, USA) from anesthetized or physically restrained tigers and lions (Figure 2 *b*) and allowed to clot at room temperature. The serum was separated by centrifugation and stored at –20°C for future use. Serum testosterone was assayed using a kit according to the manufacturer's instructions (Amersham Life Sciences, UK).

Results and discussion

Collection of semen by electroejaculation

Using the longitudinal rectal probes it was possible to induce electroejaculation in all the lions tried ($n = 7$) but was successful only in 16 out of the 21 tigers (Table 1). The inability to obtain ejaculates from 5 of the tigers was probably not related to the age of the animals as the four animals which did not ejaculate were born after June, 1990 ($n = 2$) and April, 1992 ($n = 2$) and at least 6 tigers belonging to the same age group (2 tigers born after 1992 and 4 born in 1990) ejaculated following electrostimulation. The average age of the tigers was 8.8 ± 2.7 and the age ranged from 5.4 to 15.0 years. Earlier studies on five tigers between 3 and 11 years did result in ejaculates following electro-ejaculation[7,16]. The average age of the lions was 9.3 ± 4.2 years and the age ranged from 5.1 to 15.8 years.

Semen characteristics of captive tigers and lions

Ejaculate characteristics such as ejaculate volume, per cent motile spermatozoa, per cent morphologically normal spermatozoa and sperm concentration have been routinely used to assess the overall quality of a semen sample[7,16,17]. The results indicate that the ejaculate volume, per cent motile spermatozoa and sperm concentration were less in tigers than that observed in lions (Table 2). But the

Table 2. Semen characteristics and serum testosterone levels of captive tigers and lions from three zoos in India

	Tigers ($n = 16$)	Lions ($n = 7$)
Age (year)	8.8 ± 2.7 (5.4–15.0)*	9.3 ± 4.2 (5.1–15.8)*
Semen pH	7.66 ± 0.09 (7.0–7.9)	7.85 ± 0.67 (7.4–9.8)
Ejaculate volume (ml)	1.41 ± 0.9 (0.3–3.7)	3.94 ± 2.4 (1.25–9.0)
Sperm concentration ($\times 10^6$/ml)	42.1 ± 20.2 (12–84)	52.1 ± 25.1 (20–95)
Sperm motility (%)	46.9 ± 14.9 (25–80)	63.1 ± 18.0 (35–90)
Normal spermatozoa (%)	74.82 ± 11.7 (55–93)	77.09 ± 10.4 (60–93)
Abnormal spermatozoa (%)	25.18	22.91
1. Macrocephalic	0.67 ± 0.47	0.73 ± 0.71
2. Microcephalic	0.70 ± 0.49	0.39 ± 0.29
3. Bicephalic	0.51 ± 0.75	0.48 ± 0.58
4. Tricephalic	0.02 ± 0.08	0.05 ± 0.10
5. Detached head	0.95 ± 0.08	0.87 ± 0.98
6. Amorphous head	1.69 ± 1.05	2.42 ± 2.00
7. Tightly coiled tail	15.29 ± 10.14	11.47 ± 4.80
8. Bent neck	0.83 ± 0.62	0.0
9. Bent midpiece	0.42 ± 0.16	0.57 ± 0.80
10. Bent tail	2.80 ± 1.64	4.73 ± 4.49
11. Biflagellate	0.09 ± 0.22	0.16 ± 0.15
12. Cytoplasmic droplet	0.91 ± 0.66	0.70 ± 0.66
13. Combined defects**	0.30 ± 0.08	0.34 ± 0.49
Serum testosterone (pg/ml)	1729 ± 1134 (2250–4100)	1849 ± 527 (500–5000)

*Values represent mean ± standard deviation. Values in parentheses indicate the range of variation.
**Spermatozoa showing more than one defect.

number of spermatozoa with abnormal morphology did not vary in the tigers and lions. Compared to Wildt's study on 5 tigers from Henry Doorly Zoo, Nebraska, USA[16] the tigers in the present study ejaculated very small volumes of semen and the percentage sperm motility was also distinctly decreased. But they did show an increase with respect to sperm concentration and a decrease also in the number of morphologically abnormal spermatozoa. Further, the data on lions are similar to a ten-year-old study on 8 lions from Sakkarbaug Zoo with respect to volume of ejaculate and per cent motile spermatozoa[7] but the 7 lions analysed by us exhibited an increase in spermatozoal concentration and a distinct decrease in per cent of spermatozoa with abnormal morphology. It is difficult to attribute any reason(s) for these discrepancies but more studies on similar aspects may reveal whether the variations observed are intrinsic to the animals. Further, it may also be worthwhile to ascertain whether other factors such as age, season and frequency of collection also influence ejaculate characteristics[10,18]. Our preliminary studies indicated that lions could be induced to electroejaculate all through the year but the volume of ejaculate varied and this variation could not be related to the season of the year (unpublished data).

Pleiomorphic spermatozoa of captive tigers and lions

A close relationship seems to exist between genetic diversity and sperm pleiomorphism. This conclusion is based on the observation that cheetahs, in captivity or free living, produce high proportion of pleiomorphic spermatozoa (> 60%)[16,19] and also exhibit striking genetic uniformity or increased homozygosity[5,6,20] as observed in inbred domestic cats or laboratory animals[14,15,21]. In fact to check the above assumption Wildt *et al.*[7] studied simultaneously the semen characteristics and genetic variability in three population of lions namely an outbred population in the Serengeti National Park (Africa), a second group living in the Ngorongoro Crater (Africa) thought to have originated from the inbreeding of six to fifteen original founders and a third group of Asiatic lions from the Sakkarbaug Zoo (India). The study revealed that the Serengeti group was genetically more heterozygous compared to the other two populations and the Asiatic lions were genetically monomorphic and showed significant decrease in motile spermatozoa per ejaculate and an increase in pleiomorphic spermatozoa to 66% (ref. 7). Based on these studies it was concluded that the Asiatic lion which has experienced a severe population bottleneck and has been inbreeding eversince is a highly endangered animal. However, the present study shows that the mean percentage of abnormal spermatozoa in lions was only about 23% and in the three lions from the Sakkarbaug Zoo, Junnagadh, the pleiomorphic spermatozoa was 10, 22 and 40%. In the 16 tigers from the Indian zoos, pleiomorphic spermatozoa ranged from 5% to 45% and the mean was about 25% and in the majority of the animals pleiomorphic spermatozoa was less than 20%. Wildt *et al.*[22] had observed that in tigers pleiomorphic spermatozoa ranged from 19 to 39%. Thus the situation with respect to percentage of pleiomorphic spermatozoa in Indian lions and tigers is not as alarming. Recently, Singh and his group[10] studied the genetic variation in Asiatic lions and Indian tigers and showed that the genetic heterozygosity in 38 lions and 22 tigers was 25.82% and 22.65% respectively. The same authors also showed that the genetic variability in 50 to 125-year-old skin samples from museum specimens of Indian tigers was also similar to the genetic variation in the present population (21.01%). Thus they concluded that the low genetic variability may be the characteristic feature of these species and not the consequence of prolonged inbreeding[10].

In addition to lions and tigers, pleiomorphic spermatozoa have been observed in many felids[7,16,17,22,23] and

Figure 3. Bright field photomicrographs of spermatozoa of tiger stained with papanicolau (×1000). *a*, A normal spermatozoon and two more spermatozoa with coiled tails; *b*, A macrocephalic (arrow) and a decapitated spermatozoon; *c*, A biflagellate spermatozoon; *d*, A spermatozoon with a cytoplasmic droplet; *e*, A microcephalic spermatozoon (arrow) and a spermatozoon with a tightly coiled tail; *f*, A spermatozoon with a bent neck; *g*, A spermatozoon with a partially coiled tail and a spermatozoon with an enlarged round head.

the abnormalities observed arise probably due to defective spermatogenesis (abnormalities 1 to 8 of Table 2) or defective sperm maturation process (abnormalities 9 to 13). In this study, both the tigers and lions exhibited a variety of morphologically abnormal spermatozoa (Table 2) and the predominant defects in the spermatozoa were due to coiling of the tail, bending of the midpiece and bending of the tail (Figures 3–6). Wildt *et al.*[7] had earlier indicated that in the lions of Sakkarbaug Zoo and the tigers of Henry Doorly Zoo in addition to the above three defects the presence of cytoplasmic droplet was also very high, implying that many of the spermatozoa following epididymal maturation failed to shed the cytoplasmic droplet[7,16,22]. The high incidence of pleiomorphic spermatozoa was also shown to be independent of the technique used for ejaculation and the period of ejaculatory abstinence[19,23].

Levels of testosterone in serum of captive tigers and lions

Testosterone is essential for normal spermatogenesis in mammals and reduced amounts would impair spermatogenesis. Wildt *et al.*[7] observed that in the lions of Sakkarbaug Zoo and Ngorongoro (which are inbred) which exhibited more than 50% abnormal spermatozoa the serum testosterone was three-fold low (< 1 ng/ml) compared to the outbred lions of Serengeti (~ 1.5 ng/ml). In the present study we observed that the mean serum testosterone in the lions from the Indian zoos was 1.85 ng/ml and in the tigers it was 1.72 ng/ml (Table 2) and these values are comparable to those reported by Wildt *et al.*[7,16]. In fact, the mean testosterone value of the Indian lions was very similar to that observed for the outbred lions of Serengeti[7]. The Indian lions also have a decreased percentage of abnormal spermatozoa, thus implying that they are not inbred as reported by Wildt *et al.*[7].

Hamster zona-free oocyte penetration by tiger and lion spermatozoa

Both the lion and tiger spermatozoa were capable of binding and penetrating the zona-free hamster oocytes (Table 3), with the former penetrating about 74% and the latter 87% of the oocytes respectively. The consistently higher penetration observed in the tiger may be

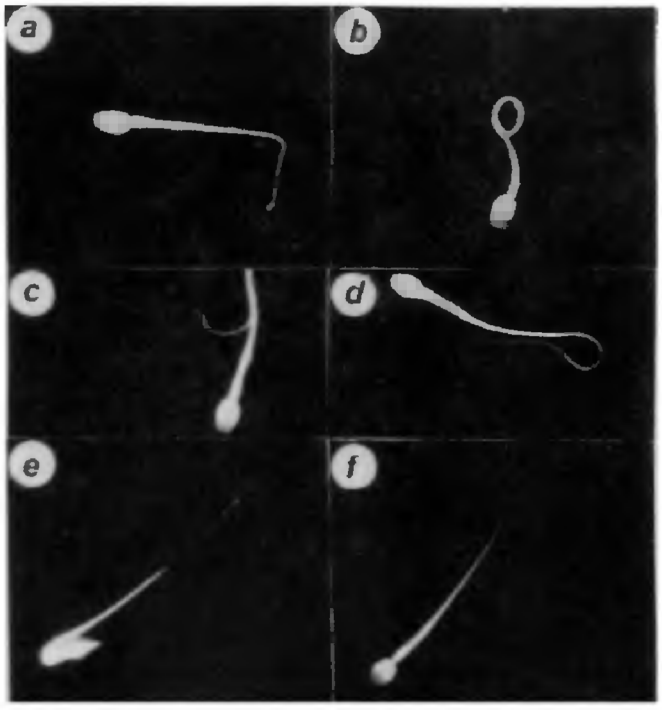

Figure 4. Fluorescence photomicrographs of spermatozoa of tiger stained with chlortetracycline (× 1000); ***a***, A spermatozoon with a bend in the end piece of the tail; ***b***, A spermatozoon with a coiled tail; ***c***, A spermatozoon with a bend just below the midpiece of the tail, ***d***, A spermatozoon with a partially coiled tail; ***e***, A spermatozoon with a pointed head and a bent middle piece; ***f***, A normal spermatozoon.

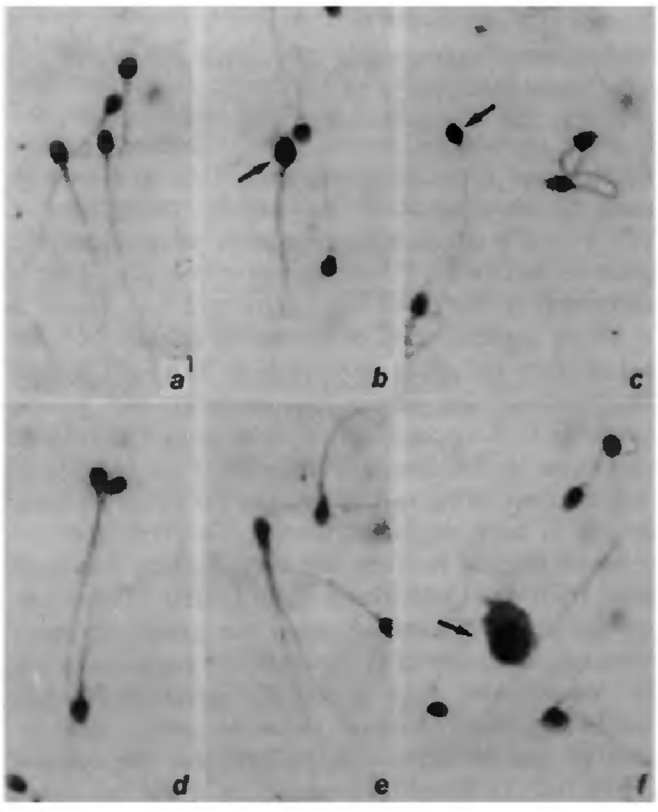

Figure 5. Bright field photomicrographs of spermatozoa of lion stained with papanicolau (× 1000). ***a***, Normal spermatozoa; ***b***, A macrocephalic spermatozoon (arrow); ***c***, A microcephalic spermatozoon (arrow) and spermatozoa with coiled tails; ***d***, A bicephalic spermatozoon; ***e***, A biflagellate spermatozoon with amorphous head; ***f***, A spermatid (arrow), a decapitated spermatozoon and a spermatozoon with a tightly coiled tail.

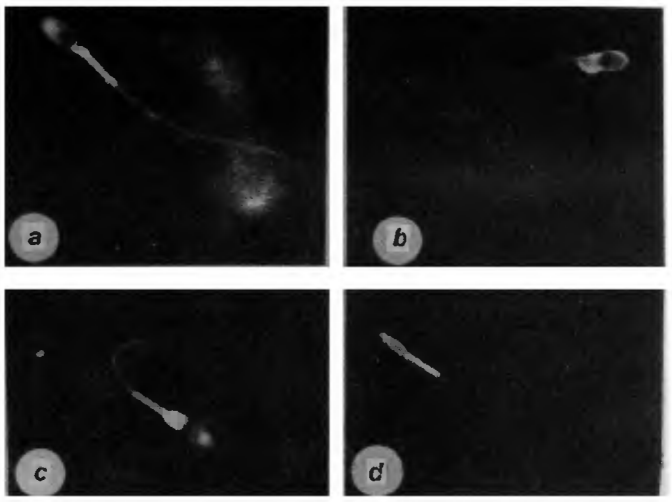

Figure 6. Fluorescence photomicrographs of spermatozoa of lion stained with chlortetracycline (× 1000). *a*, A normal spermatozoon; *b*, A spermatozoon with a cytoplasmic droplet; *c*, A spermatozoon with a coiled tail; *d*, A spermatozoon with a pointed head.

because the semen of the animals tried had very few pleiomorphic spermatozoa (10%) whereas the lion semen had about 40% pleiomorphic spermatozoa. Earlier studies had indicated that ejaculates from normospermic (< 40% abnormal sperm) domestic cats and leopard cats penetrated a greater number of zona-free hamster oocytes or zona-intact cat oocytes compared to ejaculates of teratospermic cats which had more than 60% pleiomorphic spermatozoa[24–26], thus indicating that teratospermia affects gamete interaction. IVF studies in puma and tiger also showed that teratospermia may be responsible for poor fertilizing ability of spermatozoa[27–28]. Both the neat and cryopreserved semen samples of lions and tigers successfully penetrated the zona-free hamster oocytes (Table 3, and unpublished results).

Conclusions

Wildt *et al.*[7] based on the ejaculate characteristics, levels of LH and testosterone in serum and allozyme heterogeneity studies of lions from Sakkarbaug Zoo, concluded that the Asiatic lions are highly inbred and exhibit low genetic variation. However, the results of this study on 16 tigers and 11 lions from three zoos in India clearly indicate that a good majority of the animals do not show inbreeding depression effects. In fact, in most of the animals the levels of testosterone, the spermatozoal number and percentage of motile spermatozoa was high and the incidence of morphologically abnormal spermatozoa was low (< 25%) as in outbred animals. Further, the sperms were capable of fertilizing zona-free hamster oocytes. These results confirm the findings and conclusions of Singh *et al.*[14] who, based on the observed

Table 3. *In vitro* penetration of zona-free hamster oocytes by tiger and lion spermatozoa

Animal	Nature of the semen sample	Number of oocytes used	Number of oocytes penetrated (%)	Average number of sperm per oocyte
Lion	Neat	96	73.75 ± 2.5	23.82 ± 1.01 (7–53)*
Tiger	Cryopreserved	33	87.25 ± 2.98	9.35 ± 0.83 (2–20)*

*Values represent mean ± standard deviation. Values in parentheses indicate the variation in the number of sperm that penetrated the oocytes.

genetic variation in Indian tigers and Asiatic lions, concluded that the genetic variability was a characteristic feature of these species and was not due to intensive inbreeding. The results of the present study would facilitate identification of animals with the best semen profiles and such individuals could be used in controlled breeding programmes to ensure propagation and genetic variability. It is also satisfying to know that the Indian tiger and the Asiatic lion are yet far away from the point of no return.

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Cryopreservation of semen of tigers and lions: Computerized analysis of the motility parameters of the spermatozoa

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A computer-aided semen analyser was used to monitor motility and motility parameters of tiger and lion spermatozoa prior to and after cryopreservation to ascertain changes, if any, and to establish the quality of the semen sample. The data demonstrate a consistent decrease in various motility parameters of spermatozoa following freeze-thaw and unlike spermatozoa from unfrozen semen these are slower, less progressive and their trajectories are less planar. Such data could form the basis for discerning the quality of a semen sample using data of a semen sample from a proven fertile male as the baseline data.

SEMEN banking by cryopreservation is a great boon to wildlife conservationists since such preserved semen could be used when and where needed and more importantly to improve the genetic heterogeneity in a particular species or to introduce disease-resistant genes. Though protocols for cryopreservation of cattle and human semen are very well established, it is necessary to bear in mind that cryopreservation protocols are not universally applicable to all animals and need to be modified[1,2]. These protocols vary with respect to the semen diluent used, the cryoprotectant employed, the freezing regime and also dependent on whether the storage was to be done in straws, in ampules or as pellets[3–5]. Attempts have been made earlier to cryopreserve semen from tigers[6,7] and such spermatozoa have been evaluated with respect to per cent motility and fertilizing ability. Cryopreservation is also known to decrease percentage motility, increase acrosomal loss and decrease fertilizing ability[8,9]. However, many of the above parameters for evaluating cryopreserved sperm are subjective and are not easy to carry out.

In the present study, a computer-aided sperm analyser (CASA) was used to monitor motility and motility parameters such as curvilinear velocity (VCL), progressive velocity (VSL), path velocity (VAP), straightness (STR = VSL/VAP), linearity (LIN = VSL/VCL), the amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) of tiger and lion semen samples prior to and after cryopreservation. Earlier studies have indicated that based on these motility parameters it has been possible to objectively discriminate an immature

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