Genetic diversity affects testicular morphology in free-ranging lions (Panthera leo) of the Serengeti Plains and Ngorongoro Crater

L. Munson¹, J. L. Brown², M. Bush², C. Packer³, D. Janssen⁴, S. M. Reiziss² and D. E. Wildt²

¹Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901, USA; ²Conservation and Research Center, National Zoological Park, Smithsonian Institution, Front Royal, VA 22630, USA; ³Ecology and Behavioral Biology, University of Minnesota, Minneapolis, MN 55455, USA; and ⁴Department of Veterinary Services, Zoological Society of San Diego, San Diego, CA 92112, USA

Reduced genetic variability is known to adversely affect ejaculate quality in inbred lions (Panthera leo) physically isolated in the Ngorongoro Crater compared with outbred lions inhabiting the adjacent Serengeti Plains in East Africa. This study compared the histomorphology of testicular biopsies from these two lion populations. Ngorongoro Crater lions had fewer (P < 0.05) seminiferous tubules with spermiogenesis and fewer (P < 0.05) spermatids per seminiferous tubular cross-section than Serengeti Plains lions, although seminiferous tubular diameter did not differ (P > 0.05) between populations. Interstitial areas were greater (P < 0.05) in Crater than in Plains lions, but no qualitative differences were evident, suggesting that proportionately less testicular area was occupied by seminiferous tubules in Crater lions. None of the lions in either population had evidence of testicular degeneration. Overall results suggest that inbred Crater lions have reduced spermiogenesis and less total seminiferous tubular area per testis. These data further support the premise that genetic homogeneity compromises reproductive traits in free-living, male African lions.

Introduction

Low genetic variability has been associated with poor seminal quality in lions (Panthera leo; Wildt et al., 1987), cheetahs (Acinonyx jubatus; Wildt et al., 1983; O'Brien et al., 1985) and Florida panthers (P. concolor coryi; Barone, et. al., 1994; Roelke et al., 1993). In a study of outbred lions free-ranging in the Serengeti National Park (Tanzania) and an inbred population physically isolated within the Ngorongoro Crater in the same ecosystem, there was a direct relationship between loss of genetic variability and an increasing number of structurally abnormal spermatozoa per electroejaculate (O'Brien et al., 1987; Wildt et al., 1987). Declining reproductive performance with increased inbreeding also has been detected in the Crater lion population (Packer et al., 1991). However, the mechanism by which compromised genetic variability influences these physiological processes has largely gone unstudied in this as well as other species.

In an earlier evaluation, we suspected that impaired spermatogenic function may be related to an endocrine imbalance, so basal and GnRH-stimulated LH, FSH and testosterone secretion were compared between lions living in the two locations (Brown et al., 1991). No significant differences were found in pituitary or testicular hormone production.

The present study was conducted to determine whether the histomorphological characteristics of the testes of lions with high versus low genetic diversity correlated with observed seminal differences. Testis biopsies were evaluated in detail for normal microanatomy and spermiogenesis to determine whether poor semen quality in the less genetically diverse Crater lions was caused by abnormal spermatogenesis.

Materials and Methods

Animals

Male lions were descendants of prides studied consistently since 1966 in the Serengeti Plains ecosystem (Schaller, 1972; Bertram, 1975) and since 1974 in the adjacent Ngorongoro Crater (Packer et al., 1988). The latter is an extinct volcanic caldera that restricts migration and interbreeding with lions of the nearby Serengeti Plains. All study animals were resident males of established prides, and ages of lions were known. Individual lions in both populations were identified by facial and ear scars, whisker patterns and natural markings (Pennycuick and Rudnai, 1970). All lions in this study were considered sexually mature (3.5−9.75 years of age) (Schaller, 1972; Bertram, 1975; Packer et al., 1988). None of the males had been mating on the day they were sampled.
Table 1. Histomorphometry of testses from two populations of
free-ranging lions in the Serengeti ecosystem

<table>
<thead>
<tr>
<th></th>
<th>Serengeti</th>
<th>Ngorongoro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plains lions</td>
<td>Crater lions</td>
</tr>
<tr>
<td>Number of spermatids per</td>
<td>134.6 ± 26.6(^a)</td>
<td>88.8 ± 13.8(^b)</td>
</tr>
<tr>
<td>seminiferous tubule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of seminiferous tubules with spermiogenesis</td>
<td>84.2 ± 8.6(^a)</td>
<td>67.8 ± 6.8(^b)</td>
</tr>
<tr>
<td>Number of degenerate cells per seminiferous tubule</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Seminiferous tubule diameter (µm)</td>
<td>68.9 ± 5.1</td>
<td>69.4 ± 4.8</td>
</tr>
<tr>
<td>Interstitial area (10(^4) µm(^2))</td>
<td>2.0 ± 0.3</td>
<td>2.5 ± 0.3(^b)</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

\(^a\)Within rows, values with different superscripts are significantly different (\(P < 0.05\)).

matozoa, testis volume of 94.6 ± 17.0 cm\(^3\) and a basal circulating testosterone concentration of 0.6 ± 0.5 ng ml\(^{-1}\). Calculated values (mean ± SEM) for the six biopsied Ngorongoro Crater lions were 64.6 ± 52.0 ± 10\(^6\) spermatozoa per ejaculate, 11.5 ± 15.5 × 10\(^6\) spermatozoa ml\(^{-1}\) ejaculate, 32.8 ± 8.6% normal spermatozoa, testis volume of 62.9 ± 26.8 cm\(^3\) and a basal circulating testosterone concentration of 0.74 ± 0.5 ng ml\(^{-1}\). Differences in testes volumes and proportions of normal spermatozoa (\(P < 0.05\)) between Crater and Plains lions that were previously reported (Brown et al., 1991) were maintained in these subpopulations of biopsied lions.

Statistical analysis

Mann–Whitney two sample tests were used for comparing seminiferous tubule diameter, number of spermatids, numbers of degenerate cells and interstitial areas between locations. Relationships between reproductive variables were determined by least squares linear regression analyses. Data are presented as means ± SEM.

Results

Active spermiogenesis was evident in biopsies from all lions sampled in the Ngorongoro Crater and Serengeti Plains populations, and all lions had mature spermatids. Although seminiferous tubule diameters did not differ (\(P > 0.05\)) between populations, Crater lions had fewer (\(P < 0.05\)) seminiferous tubules with spermatids compared with their Plains counterparts (Table 1). Crater lions also had fewer (\(P < 0.01\)) spermatids per seminiferous tubular cross-section than did Plains lions (Fig. 1). For both populations, the number of spermatids per tubular cross-section did not correlate with the number of degenerate cells per tubule (\(r = 0.43\); \(P > 0.05\)), total sperm numbers per ejaculate (\(r = 0.56\); \(P > 0.05\)), or the mean seminiferous tubular diameter (\(r = 0.47\); \(P > 0.05\)). Furthermore, there was no correlation between combined testis volume and the seminiferous tubular diameter (\(r = -0.06\); \(P > 0.05\)). Although spermatid numbers were lower in Crater lions, the...
Fig. 1. Photomicrograph of adult lion testes from (a) the Ngorongoro Crater and (b) Serengeti Plains. Crater lions have fewer spermatids per seminiferous tubule than do Serengeti Plains lions, although mean tubular diameters did not differ between populations. Masson’s trichrome stain. Scale bars represent 10 µm.

number of degenerate cells per seminiferous tubule did not differ between populations. Specific morphological defects of ejaculated spermatozoa, such as coiled flagellum or bent midpiece with droplet (Brown et al., 1991), were not identifiable in tissue sections.

Mean interstitial areas including Leydig cells were proportionally greater ($P < 0.05$) in Crater than in Plains lions (Table 1). There was no correlation between the combined testis volume and the interstitial area ($r = -0.32; P > 0.05$). Leydig cells (interstitial cells) were arranged in clusters of five to >50 cells in the intertubular interstitium in both populations. Some, but not all, Leydig cell clusters surrounded blood vessels. No morphological differences were observed between the Leydig cells of Plains and Crater lions or between lions with high and low basal testosterone concentrations. The width of the interlobular septa ranged from 10 µm to 70 µm within individual biopsies. Some degree of interstitial variation within and among lions was caused by biopsy-induced oedema and haemorrhage and regional clustering of Leydig cells. No thickening of peritubular basement membranes or abnormal matrix deposits in the lamina propria, characteristic of testicular degeneration or hypoplasia, were noted.

**Discussion**

Previous comparisons of these two populations of free-ranging lions in the Serengeti ecosystem measured decreased sperm motility and markedly higher proportions of structurally abnormal spermatozoa in electroejaculates of the more genetically monomorphic Ngorongoro Crater lions (Wildt et al., 1987; Brown et al., 1991). Our previous analyses of circulating gonadotrophin (LH and FSH) and testosterone concentrations revealed that sperm quality differences between locations is unrelated to endocrine dysfunction (Brown et al., 1991). The present study took a more direct approach by assessing
testicular structure and spermatogenesis. Total spermatogenesis per tubular cross-sectional area was comparable between the lions living in these two locations, because seminiferous tubular diameters were similar. However, morphometric analyses of testicular biopsies indicated that the Crater lions produced fewer spermatids than did Plains lions. Also, Crater lions had greater interstitial areas and lower testicular volumes than did Plains lions, suggesting that proportionately less testicular parenchyma was occupied by seminiferous tubules in Crater lions. Taken together, these findings could account for the reduced sperm production in the ejaculates of Crater lions (Brown et al., 1991).

In other species, seminiferous tubular diameter is diminished if fewer spermatogonia undergo spermatogenesis or if spermatogenic arrest occurs during early stages (de Kreter and Kerr, 1988; McEntee, 1990; Trainer, 1992). This implies that reductions in spermatid numbers in Crater lions occurred during terminal stages of differentiation (spermiogenesis) and not from spermatogonia or spermatocyte loss. All stages of spermatogenesis were observed in both populations, but direct quantification of the specific stages was not possible for this study because cell morphology was distorted by crush artifacts. In our study, direct quantification of spermatid numbers appeared to provide a more reliable index of testicular function (Berdton, 1989) than did seminiferous tubular diameter, which is used in other species (Krishnalingham et al., 1982; Amann, 1986). Indeed, seminiferous tubular diameter did not correlate with lower spermatid production in Crater lions, suggesting that it may be an unreliable index of testicular function when spermatogenic rates are normal but terminal differentiation is compromised.

Normal maturation and morphogenesis of spermatozoa are under inherent genetic controls with epigenetic modification by Sertoli cells (Bardin et al., 1988; de Kreter and Kerr, 1988). Sperm cell loss can result from intrinsic lethal genes or a lack of paracrine support during development (Bardin et al., 1988; McEntee, 1990). An inherited developmental block, such as the spermatid-to-spermatozoal arrest reported in hypospermic bulls (McEntee, 1990), is unlikely to be the basis of low spermatid numbers in Crater lions, because all stages of spermatogenesis (including late spermatids and spermatozoa) were found. Crater lion ejaculates also contained high proportions of spermatozoa with defects occurring from abnormal maturation in the seminiferous epithelium, such as coiled flagellum and mid-piece abnormalities (Wildt et al., 1987; de Kreter and Kerr, 1988; Brown et al., 1991). Thus, lower spermatid numbers in seminiferous tubular cross-sections of Crater lion testes may indicate that developmentally defective spermatozoa are released prematurely from Sertoli cells.

Testicular volumes tended to be smaller in Crater than Plains lions, but size disparities and seminal quality (Brown et al., 1991) were not reflected in reduced seminiferous tubular diameter. Smaller testes size in Crater lions may have been caused by fewer or shorter seminiferous tubules because (1) the major determinants of testicular volume are seminiferous tubule diameter, number, and length (Mori and Christensen, 1980; Setchell and Brooks, 1988), (2) Ngorongoro Crater lions had normal seminiferous tubular diameters and (3) Crater lions had relatively more testicular area occupied by interstitium than Plains lions. No Crater lions had lesions typical of testicular degeneration, such as thickening of the basement membrane or increased interstitial matrix (McEntee, 1990), to account for this smaller testes size. Also, interstitial area did not correlate with testicular volume, further indicating that total seminiferous area must be the main determinant of testis volume. If the assumption that Crater lions had fewer or shorter tubules is correct, then reduced total seminiferous tubular surface area would result in less total sperm production in Crater lions. Because lions normally copulate frequently (Seager and Demorest, 1978; Packer and Pusey, 1983), low sperm reserves may be a contributing factor in the decline of Crater lion numbers (Packer et al., 1991). Although small tissue samples precluded the assessment of seminiferous tubular numbers and lengths in this study, future evaluations could include this parameter.

These morphological findings confirm previous functional data indicating that genetic diversity influences reproductive characteristics of free-ranging lions (Wildt et al., 1987; Brown et al., 1991; Wildt, 1994). Assuming that the data from the genetically diverse lions of the Serengeti Plains represent the norm for this species, then it is apparent that loss of genetic diversity in Crater lions has profound effects on spermatic numbers, testis volume and overall ejaculate quality. Because these are nonlethal traits, they will be perpetuated in the population and more highly expressed as the population diminishes. The reduced testicular function in the homogenetic population of Crater lions may be adequate to maintain the population under normal conditions, but provides limited reserves during periods of environmental stress, disease epidemics or other catastrophic events.

The authors thank A. Pusey, S. O'Brien, D. Gilbert and S. Monfort for assistance with sample collection. They also thank K. N. Harji, Coordinator of the Serengeti Wildlife Research Institute and the Government of Tanzania for support. This study was supported, in part, by grants from The National Geographic Society, Friends of the National Zoo and the National Science Foundation (No. 8507087).

References

Amann RP (1986) Detection of alterations in testicular and epididymal function in laboratory animals Environmental Health Perspectives 70 149–158
Krishnalingham V, Ladds PW, Entwistle KW and Holroyd RG (1982) Quantitative macroscopic and histological study of testicular hypoplasia in Bos indicus strain bulls Research in Veterinary Sciences 32 131–139


