REPRODUCTION IN THE ZEBRA STALLION (*EQUUS BURCHELLI ANTIQUORUM*) FROM THE KRUGER NATIONAL PARK

G. L. SMUTS
National Parks Board of Trustees, Skukuza

ASTRACT

Reproductive characteristics of free-ranging zebra stallions are described using data collected from 270 cropped specimens and field observations of live animals. Socially zebra populations comprise coherent family groups dominated by a single stallion and consist of 2–11 individuals including foals. Excess stallions remain solitary or form stallion groups of two to seven individuals. The youngest stallion leading a family group was four years old. On average stallions attain psychological maturity at four and a half years of age, the pubertal interval ranging from age two to four and a half years. The youngest specimen with epididymal spermatozoa was two years old but generally this occurs at three and a half years but behavioural mechanisms prevent mature sub-adult stallions from mating with oestrous mares. Variation in testicle mass and seminiferous tubule diameter indicates that adult stallions have a reproductive peak during the summer (wet season) which coincides with the cycle of the mare. Genital abnormalities included two instances of unilateral testicular hypoplasia and one of bilateral cryptorchidism. Only the latter caused sterility.

INTRODUCTION

Reproductive characteristics of the zebra stallion were studied as part of a detailed investigation into growth and population dynamics of Burchell's zebra (*Equus burchelli antiquorum*) in the Kruger National Park, South Africa (Smuts 1974a). The purpose of the investigation was to collect data on the pubertal interval of the wild zebra stallion; age at sexual maturity; the seasonal reproductive cycle of adult animals and the incidence of reproductive abnormalities. Behavioural characteristics pertaining to reproduction were also noted.

Analysis of data has indicated that in terms of general reproduction the Burchell's zebra stallion is similar to domestic equids; sexual development, however, appears to be retarded, with wild stallions commencing to breed at about four and a half years of age.

METHODS

Between May 1969 and November 1972 the reproductive tracts of 270 zebra stallions shot in the Central District of the Kruger National Park (KNP) were examined. Since cropping was undertaken during the day it was also possible to ascertain the social status of most of the animals, *i.e.* whether they were lone stallions, members of stallion groups or leader stallions from family groups. In the short time available body measurements were taken, ages assessed (Smuts 1974b) and testes removed together with their epididymides. Each testicle was marked left or right and

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a sperm smear made by slitting the tail of the epididymis and running a glass slide over the cut ends of the epididymal ducts. Testicular tissue was taken from the centre of one testicle and fixed in 10 per cent formalin for histological examination. Three to six adult stallions (5–20 years old) were sampled per month while similar specimens were taken from younger stallions wherever they were available. The mass of each fresh testicle with its epidymis attached was determined using an electric balance. Formalin-fixed material was later dehydrated in alcohol, cleared in chloroform and embedded in paraffin wax; sections 4 to 7 μm thick were stained with Meyer's haematoxylin and counter-stained with eosin (HE). Masson's trichrome technique was also used (Culling 1957). Since all specimens were treated in roughly the same way, shrinkage due to fixation, dehydration, clearing and/or embedding was ignored.

Sperm smears were fixed in methanol and stained with 10 per cent Giemsa. Seminiferous tubule diameter was determined for each specimen by calculating the mean of 25 tubules measured in cross-section.

Between 1969 and 1972 counts of zebra were undertaken from a vehicle. These counts (Smuts 1974a) sampled the entire range of the Central District's zebra population with sex ratio and group sizes (family and stallion groups) being recorded.

In the present study puberty in the zebra stallion is regarded as the interval of time during which reproduction is possible, but terminating when full reproductive capacity or sexual maturity is reached (Emmens 1969). The period will therefore be slightly shorter than that defined by Skinner (1969), where it is stated that the onset of male puberty coincides with the time when the testes become androgenically active and the accessory glands begin to secrete fructose and citric acid, since spermatozoa appear some time after this.

RESULTS

Social organization

Observations on *E.b. antiquorum* indicate that social organization is similar to that observed in *E. b. böhmi* from East African populations which consist of coherent family and stallion groups (Klingel 1965). Family groups comprise one adult stallion (leader stallion) and one to five mares and their foals. Surplus stallions form bachelor groups or remain solitary. Mean family and stallion group sizes for the Central District are given in Table 1. On average 17,1 per cent of the population are members of stallion groups. Here a sample of 4 070 zebra were counted from a total population of 9 266 (Smuts 1972). The adult sex ratio (3–20 year-olds) for the Central District population was 1♂: 1,35♀, the sample being 3 327 zebra. This is a very significant deviation from a 1:1 ratio (Chi-square = 63,88; $P < 0{,}005$). The age distribution of 52 stallions selectively shot from family groups (*i.e.* leader stallions) is given (Table 2). From a sample of 20 young stallions (colts) selectively shot from family and stallion groups it was found that the oldest colt in a family group was 3½ years old and that the youngest member of a stallion group was nine months old. Fighting amongst adult stallions is frequently seen and is usually associated with a young mare coming on heat for the first time. Competition between stallions from both family and stallion groups for young mares in oestrus is high. Mating between stallions and mares from different groups does not, however, take place and there is thus no competition by fighting for adult mares (Klingel 1969a). In a total of 1 689 family groups counted throughout the KNP and broken down into their components (adult stallion; adult mare(s); sub-adults; immatures and foals), only three instances of mares being on their own were recorded. In one of these the stallion had been killed by lions 500 m away. Other instances of behaviour linked to reproduction include penis-flexing (incomplete masturbation) and the voidance of faeces or urine on top of these same fresh products of another zebra – particularly mares.

Puberty and sexual maturity

The curve for growth in mass versus age for zebra testes is illustrated in Figure 1. Testes of adult stallions (5–20 years old) have a mass 8–10 times greater than those of a two-year-old, while the penis is 10–15 cm longer. The mean mass of the left-plus-right testes and their epididymides for adult stallions is 302 g ($n = 195$; range = 175–465 g). At 4–4½ years of age this mass is 229 g ($n = 16$; range = 100–350 g). In 80 per cent of all adult stallions development of the left testicle exceeds that of the right. This is a highly significant deviation from the expected 1:1 ratio (Chi-square = 67,8; $P < 0{,}005$; $n = 195$ adult stallions).

Data on seminiferous tubule diameter for 32 young stallions (six months to four years old) and 61 adult stallions are illustrated in Figure 2. On the right hand side of Figure 2 the state of spermatogenesis relative to tubule diameter is also illustrated. Although spermatozoa may be present in the seminiferous tubules of zebra by the age of 2½–3 years, the first appreciable numbers were picked up in epididymal sperm smears at 3½ years of age. In one case a two-year-old stallion had sperm present in the epididymis, yet the seminiferous tissue sections were negative. This was the youngest animal in which mature germ cells were found.

Figure 3 is a scatter diagram illustrating the type of correlation existing between tubule diameter and mass of paired testes. Here standard linear regression equations and correlation

TABLE 1

Mean family and stallion group sizes for zebra from the Central District of the Kruger National Park.

	Number zebra	Number groups	Group size			
			Maximum	*Minimum*	*Mean*	*S.D.*
Family groups	4 772	1 067	11	2	4,47	1,49
Stallion groups	1 011	355	7	1	2,85	1,39

coefficients have been calculated for stallions with testis masses of (i) < 190 g ($n = 34$) and (ii) > 190 g ($n = 56$). For (i) the r-value was 0,874 ($t = 10,2$; P < 0,001) and for (ii) it was 0,227 ($t = 1,71$; P > 0,05).

To summarize the relevant data on sexual development in the zebra stallion Figure 4 is given. The curve was plotted using age-specific testis and body mass data while sexual behaviour was taken into account in data given on the right hand side of the curve (see preceding section).

TABLE 2

Age distribution of 52 Burchell's zebra leader stallions selectively shot from family groups in the Kruger National Park.

Age (years)	*Number in each age class*	*Per cent in each age class*
3	0	0
4	1	1,92
*4½	2	*3,85
5	4	7,69
6	5	9,62
7	4	7,69
8	5	9,62
9	5	9,62
10	5	9,62
11	5	9,62
*12	7	*13,46
13	2	3,85
14	0	0
15	2	3,85
16	2	3,85
17	2	3,85
18	1	1,92
19	0	0
Total	52	100,03

*4½ to 12-year-olds make up 80,8 per cent of the total.

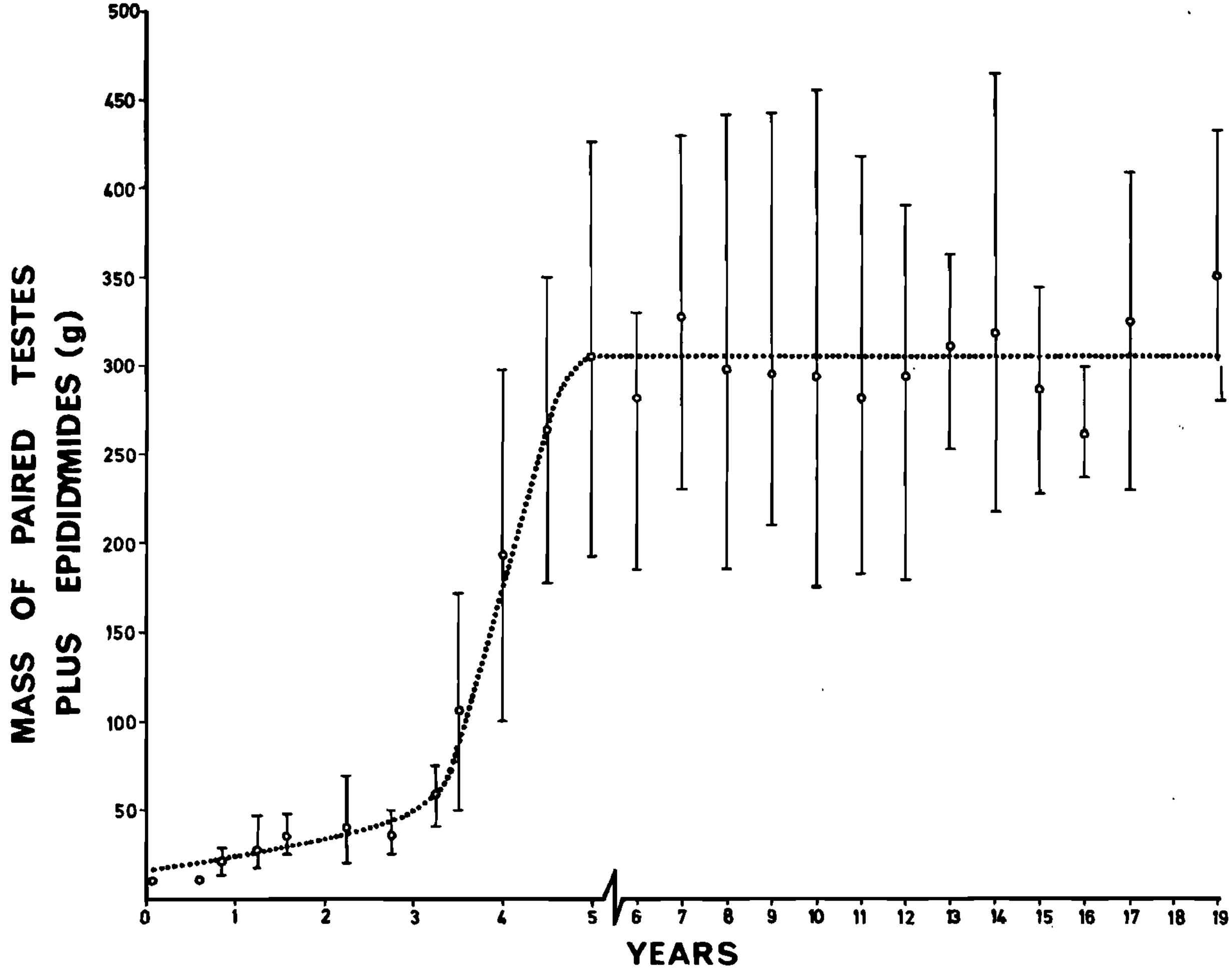

FIGURE 1

Growth in mass of Burchell's zebra testes (n = 270 stallions). Total variation indicated by the vertical lines. The curve was fitted by eye.

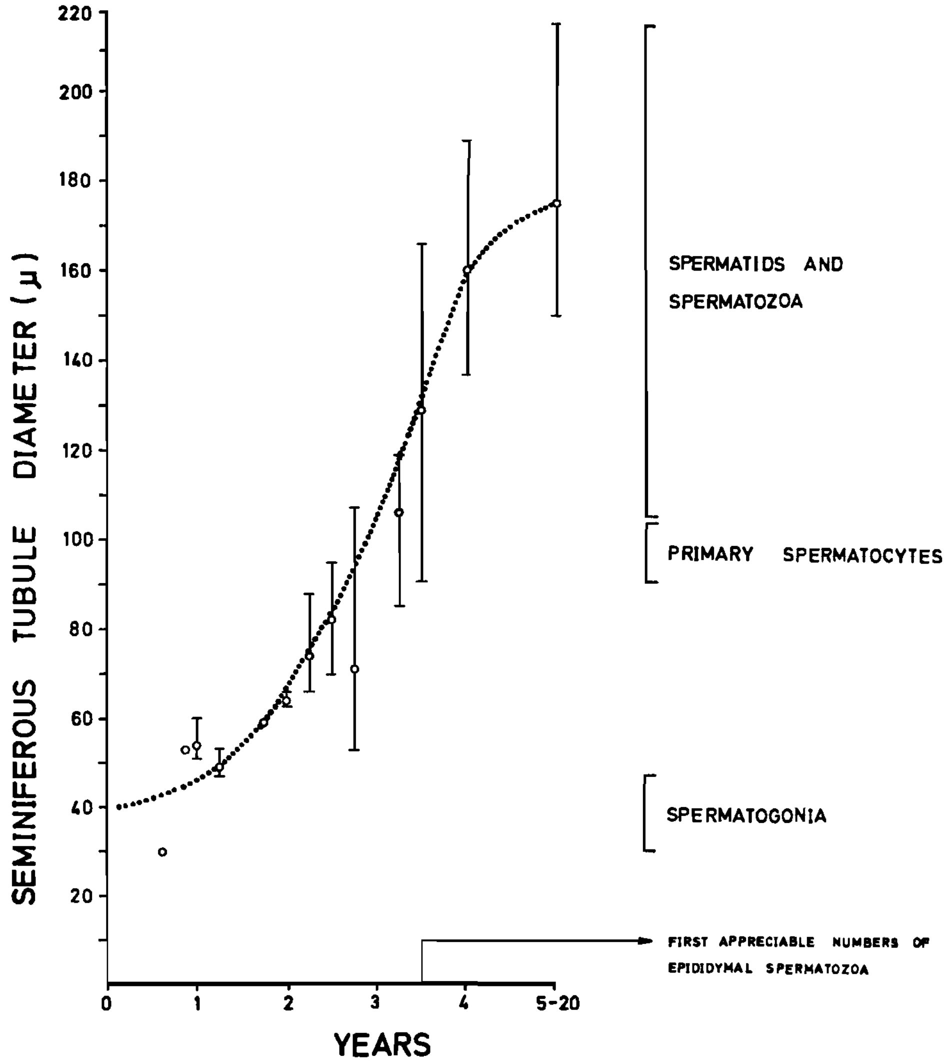

FIGURE 2

Growth of the seminiferous tubule in Burchell's zebra (n = 93 stallions). Total variation indicated by vertical lines. The curve was fitted by eye.

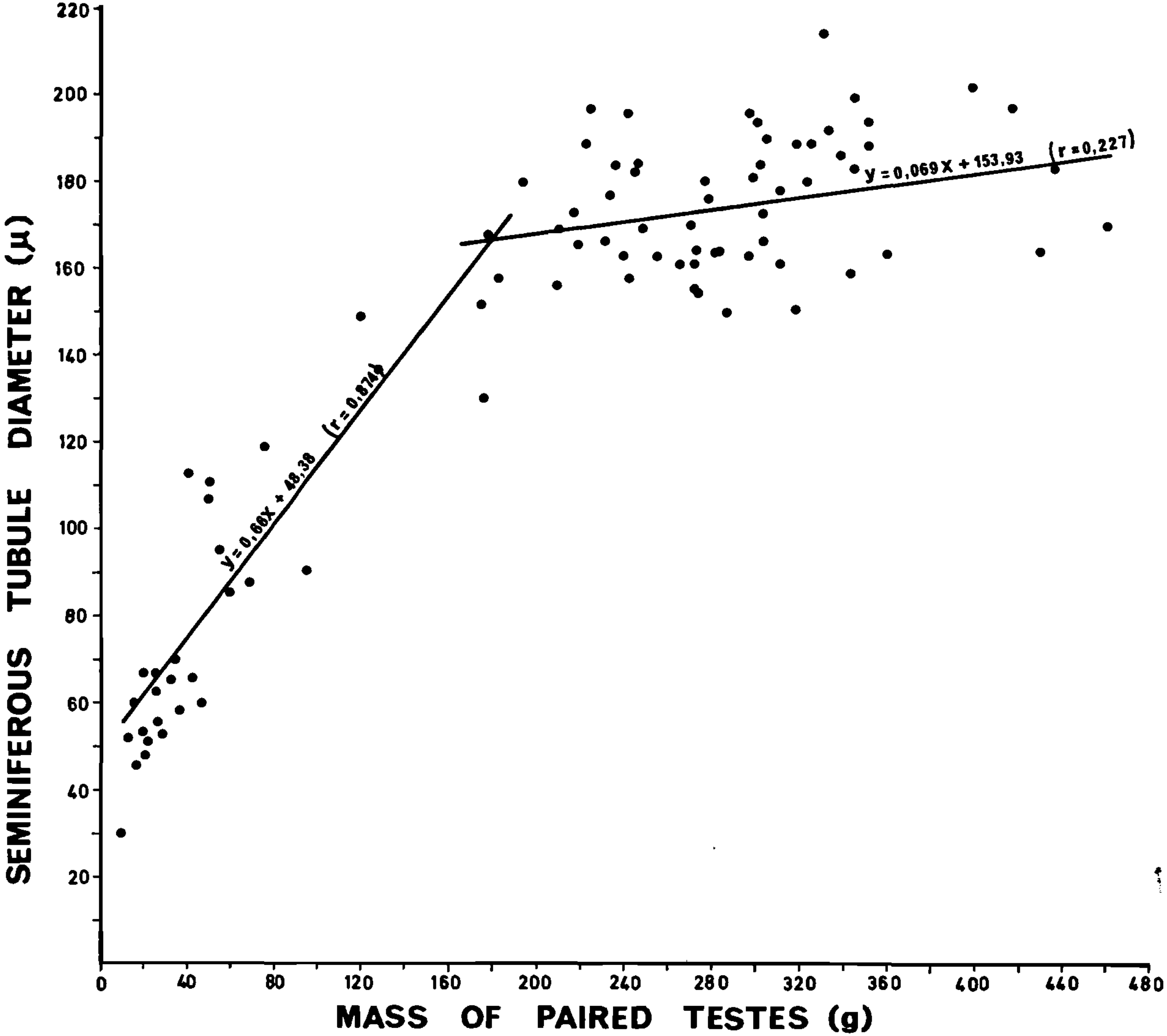

FIGURE 3

Scatter diagram of mean seminiferous tubule diameter plotted against testis mass for 87 Burchell's zebra stallions. The lines are the linear regressions with their equations for stallions with masses of paired testes, (i) < 190 grams and (ii) > 190 grams. Correlation coefficients (r) for each equation are also given.

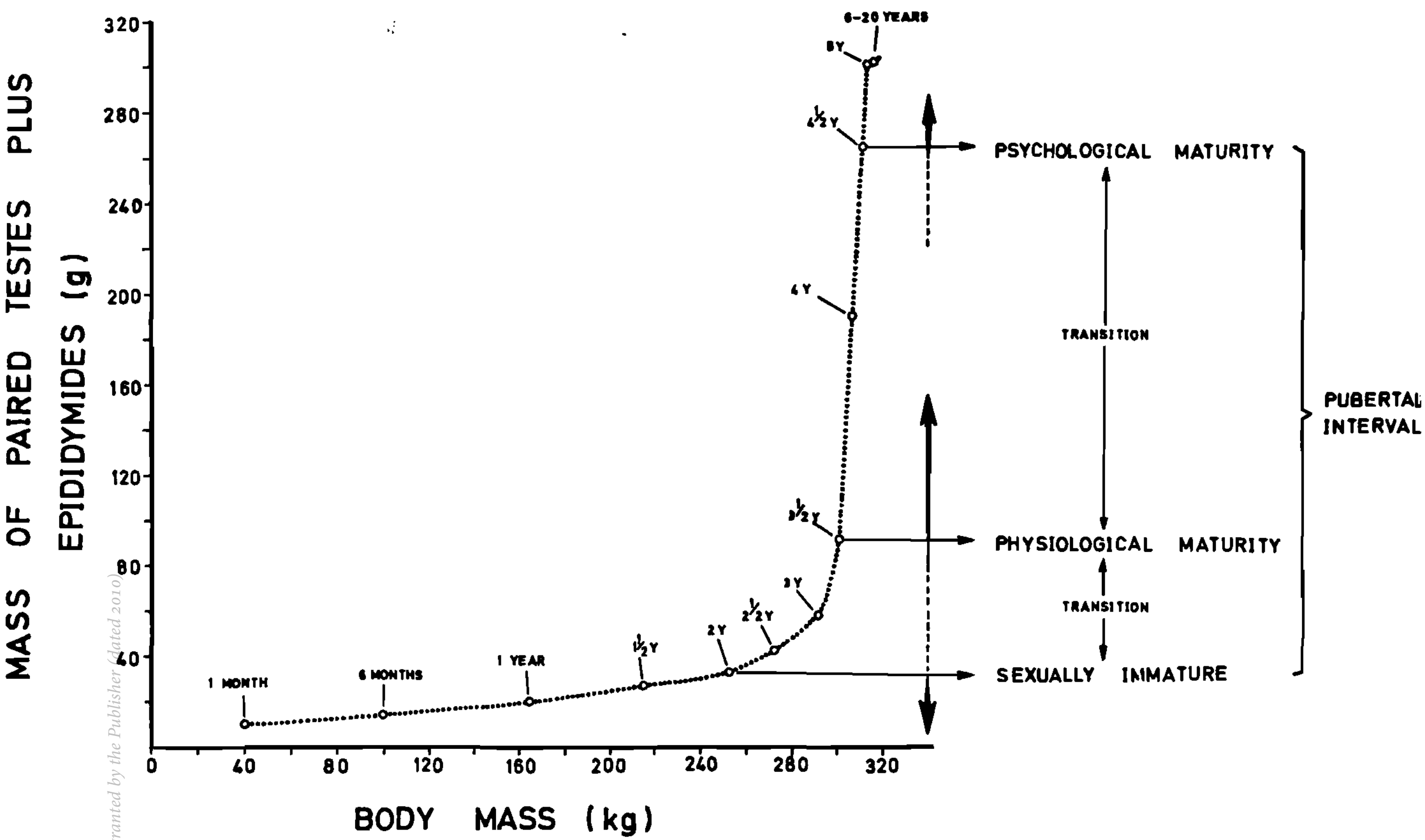

FIGURE 4

Average relationship between body mass and testis mass for Burchell's zebra. Data on puberty and maturity on the right hand side of the graph summarize average sexual development and bring it in line with growth of the testes and increase in body mass.

Seasonal reproductive activity

Figure 5 illustrates seasonal variation in testicular mass and seminiferous tubule diameter for adult zebra stallions. Additional data on tubule diameter are given in Table 3. The data on testis mass cover a three-year period (July 1969 to November 1972) and the histogram thus represents a summation of corresponding months data for the three years. Comparing zebra seminiferous tubule diameter for the winter months (April to September) with those for the summer months (October to March) by means of a t-test, a highly significant difference is obtained at the 1 per cent level ($t = 4{,}333$).

Testicular pathology

During the study three instances of gross male reproductive abnormalities were recorded in the sample of 270 tracts examined (1,1 per cent). These included two cases of unilateral testicular hypoplasia and one of bilateral cryptorchidism (see p. 222 of this issue).

TABLE 3

Seasonal variation in seminiferous tubule diameter for 50 adult Burchell's zebra stallions (5–20 years old).

Month	n	*Seminiferous tubule diameter (μm)* Maximum	Minimum	Mean	S.D.
August 1969	4	184	164	170	9
September	4	170	166	167	2
October	4	182	158	175	11
November	3	202	164	183	19
December	4	196	178	187	8
January 1970	2	196	195	196	1
February	5	194	180	187	6
March	3	188	155	175	18
April	6	184	163	174	9
May	5	196	150	168	19
June	5	173	158	166	7
July	5	186	158	170	11

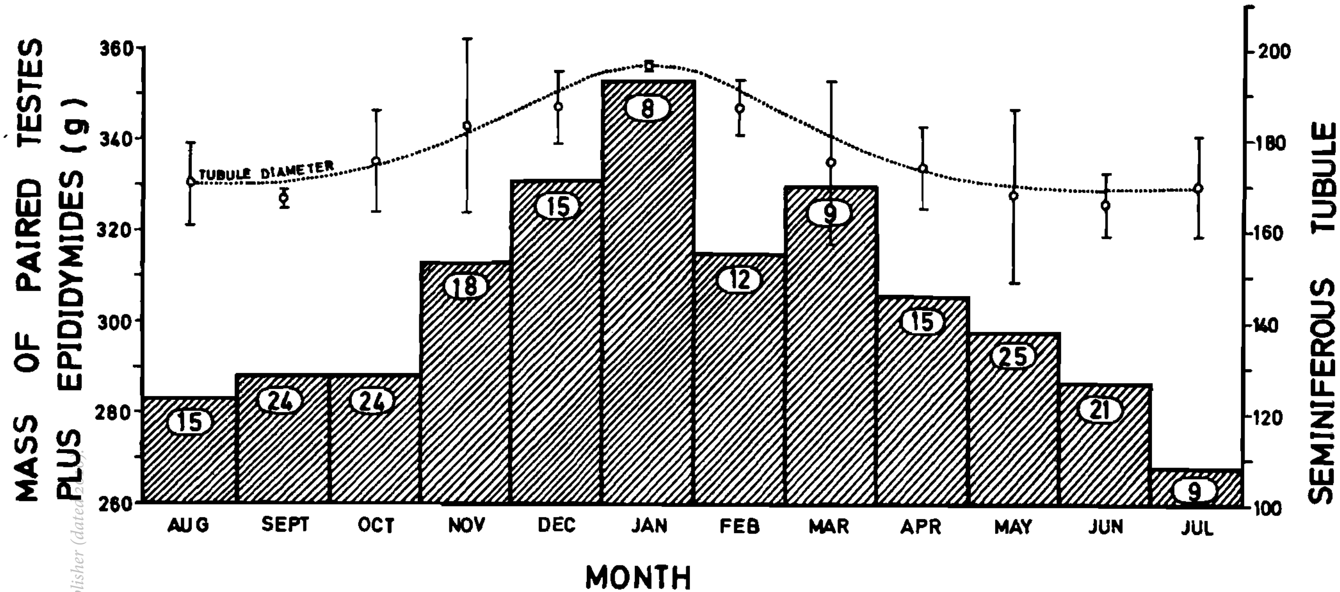

FIGURE 5

Seasonal variation in zebra testis mass (n = 195) (histogram) and seminiferous tubule diameter (n = 50) (graph) for adult Burchell's zebra stallions (5–20 years old). The number of specimens used to determine monthly testicular mass is illustrated in the histogram. Standard deviations for mean monthly tubule diameters are indicated by the vertical lines. The curve was fitted by eye.

DISCUSSION

As in most polygamous mammals the zebra stallion is relatively dispensable and in terms of reproductive numbers is subsequently less important than the mare. In this context the adult stallion is more vulnerable to predation than the mare (Smuts 1974a), thereby possibly filling an important role in perpetuation of the species. This would not have been the case had both sexes displayed the same mortality patterns. The adult sex ratio (1♂:1,35♀) also supports a differential mortality rate since sex ratio at birth and even up to two years of age, is parity (Smuts 1974a).

The social organization of Burchell's zebra is significant since the semi-isolated family groups, typically dominated by a single adult stallion, fosters interbreeding (in contrast to inbreeding), while outbreeding is not prevented. In reality the whole pattern of social behaviour promotes outbreeding (Klingel 1969a) since adolescent mares are abducted by other stallions while colts leave their family groups on or before attaining sexual maturity. In terms of population genetics the cohesive breeding unit of the zebra type may be regarded as being the optimal kind (Wright 1950). The degree to which social behaviour in the zebra promotes outbreeding is supported to some extent by the work of Osterhoff (1966) on biochemical polymorphism in the Equidae. When compared to the buffalo (*Syncerus caffer*) and the elephant (*Loxodonta africana*) (Osterhoff, Young & Ward-Cox 1972), the zebra also shows a much lower degree of inbreeding.

From Table 2 it can be seen that the youngest stallion leading a family group was four years old and that 80,8 per cent of all family group stallions were between 4½ and 12 years of age. Klingel (1965, 1969 a & b) found that the two youngest stallions (*E. b. böhmi*) starting family groups of their own were five to six years old. His study, however, was undertaken in the Ngorongoro Crater (East Africa) which has a density of zebra nine times greater than that in the Central District of the KNP. Under these conditions it is possible that competition for mares would be higher and the psychological maturity would occur later in life.

Table 2 also indicates that the prime breeding stallions are under 12 years of age. However, breeding does not stop in the older age classes as suggested by King (1965) and there could be stallions over 18 years of age still leading family groups. (Maximum ecological longevity = 21 years: Smuts 1974b). Klingel (1965) found that the oldest colts in family groups were about three years old, but mentions one case of a young stallion remaining with his group up to the age of 4½ years (Klingel 1969a). According to Klingel (1969a) the age at which a young stallion leaves his family group depends mainly on the composition of the group, most colts leaving between the ages of 12 and 25 months, after their mothers had given birth to a new foal. The fact that young stallions may remain in the family group up to the age of 3½ years ties in well with the minimum age of psychological maturity of four years (Table 2).

The lowest combined testis mass for a zebra stallion (Figure 1: 10 year-old class) of 175 g is exceeded for the first time by young stallions from the four-year-old group, indicating that full maturity is attained after this age. It should also be noted that from approximately 3½ years of age testicle mass increased rapidly with the adult mean (302 g) being reached at five years of age.

The disparity between the mass of left and right testes has also been reported for the domestic horse (Nishikawa & Hafez 1962).

There is a fairly constant increase in tubule diameter in Burchell's zebra (Figure 2) but the phenomenon is not as sudden as in the case of testis mass (Figure 1). The sigmoid curve is typical for mammals (Dryden 1969) and indicates that the attainment of puberty is a gradual process with tubular development starting before the animal is one year of age and continuing until adulthood.

The finding that certain epididymal smears showed the presence of spermatozoa while histological sections from the testis itself were negative, can be explained by the spermatogenic process which does not proceed at the same rate throughout the whole testis. When scanning large sections under the microscope it is also clear that in certain regions tubule diameters are larger and spermatogenic activity more advanced than in other regions.

Figure 3 indicates that tubule diameter initially increases proportionately to an increase in mass of the testes but that at a mass of about 190 g the relationship breaks down and tubule diameter remains relatively constant as mass of the testes continues to increase. The first regression line with its good correlation of 0,874 and the second line with the low *r*-value of 0,227 show that at about 180 g (their intercept) zebra stallions attain maturity. From Figure 1 it can be seen that on average the 180 g limit is attained at four years of age.

Figure 4 shows that puberty is represented by an interval ranging from two years of age, when the first sperms were observed in the epididymis, to 4½ years of age when full reproductive capacity and psychological maturity are attained. The data agree well with those obtained on Grant's or the plains zebra (*E. b. böhmi*). Wackernagel (1965), for example, mentions the case of a young stallion in the Basle zoo copulating for the first time at 18½ months of age, while King (1965) states that a captive two-year-old stallion showed libido before spermatogenesis was complete. Trumler (1958) observed that captive animals were physiologically mature at three years of age, while Klingel (1965, 1969a) mentions that in the wild the youngest family stallions were five to six years old.

Compared to the domestic horse sexual development in the zebra stallion proceeds at a relatively slow pace. Skinner & Bowen (1968) found that the mean age at appearance of spermatozoa in the Welsh stallion was 13 months, while similar results were obtained by Horie & Nishikawa (1955) in the Anglo-Norman breed. Nishikawa & Hafez (.962) state that sperm is first produced in the testes of the horse at one year of age and that stallions may attain sexual maturity during their second year.

From Figure 5 there appears to be little doubt that the adult stallion shows a greater degree of reproductive activity during the summer months and in particular during January. This coincides with the reproductive activity of the mare where 85 per cent of all foals are produced during the summer months (Smuts 1974a), the peak being January. Since the gestation period is just over one year (Wackernagel 1965; Klingel 1969a), peak mating activity must also occur during the summer months and, in particular, during January.

With an average testicle mass difference of 86 g between January and July (345–268 g) it can be said that testicle mass increases by 24 per cent from the months of lowest breeding activity to the peak month. During no month, however, is there complete absence of spermatogenesis. In addition to the 24 per cent increase in testicular mass, seminiferous tubule diameter increases by 13 per cent between July and January. When these results are compared to those obtained from strongly seasonal breeders such as the blesbok, *Damaliscus dorcas phillipsi* (Skinner & Huntley 1971), and the impala (Skinner 1971), where testis mass increases by 50 per cent and tubule diameter by about 33 per cent, it becomes clear that the most marked changes may be expected from the more strongly seasonal species.

Although tubule diameters differ significantly during the winter and summer months, it is obvious that the zebra stallion is not as strongly seasonal as some game animals and that reproductive activity continues throughout the year. This is supported by the young foals which can be observed during any month, while in an animal such as the impala, by contrast, all lambing (and consequently mating) occurs within a period of about two months or less (Fairall 1971). The seasonal breeding activity of the wild zebra stallion and synchronization with the cycle in the mare are phenomena which have also been observed in domestic horses (Nishikawa & Horie 1952; Nishikawa & Hafez 1962; Zemjanis 1970).

Although the stallion displays a clear reproductive cycle, it would be difficult to determine which environmental factors were playing a role in effecting the synchronization. The reason is that peak reproductive activity occurs during the periods of peak daylight hours, temperature, humidity, rainfall and consequently green vegetation (Smuts 1974a). Any one of these factors, and probably all, could play a role in the cyclic reproductive phenomenon. It has, however, been demonstrated that not only is photoperiod of great importance in the attainment of puberty in the domestic horse (Skinner & Bowen 1968), but that sexual behaviour and semen characteristics may be related to seasonal fluctuations in day length, and these can be controlled artificially to some degree (Nishikawa & Hafez 1962). Photoperiod is probably of similar importance in the seasonal cycle of the related zebra.

Since the adult stallion is sexually active throughout the year with only a slight increase during the summer months, his cycle is probably less significant than that of the adult mare. However, since peak reproductive activity in the two sexes coincide (Smuts 1974a) energy is saved and mating and parturition can take place during the optimal time of the year, *i.e.* during the summer months when food and water are plentiful.

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