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Abstract: Lifetime semen production data provide valuable insight into a species' natural history and biology as well as information about the potential fertility of males at various life stages. An understanding of the ages of sexual maturity, peak sperm production, and gonadal senescence will contribute to the design of species management plans for captive, free-ranging, and reintroduced populations of exotic animals. To describe these life stages in the cheetah, semen was collected biweekly for 13 years from a solitary male beginning at 3 years of age. The 338 ejaculates were obtained non invasively by artificial vagina. Ejaculate volume and sperm motility score, concentration, and normal morphology were recorded. A sperm quality index incorporating all five semen parameters was calculated to facilitate ejaculate comparisons. Polynomial regression analysis revealed a significant effect of age on volume, which increased throughout the animal's lifetime except for a reduction between ages 10 and 12. Concentration was also significantly affected by age and increased from the age of 3 to the age of 10, then decreased. The sperm quality index revealed a significant effect of age as it increased from age 3 to age 8, then declined as the male grew older. The cheetah did not reach peak semen production until age 8 and continued to produce good quality semen for several more years. These data were somewhat unexpected, given the average cheetah life expectancy of approximately 7 years.

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Lifetime Semen Production in a Cheetah (*Acinonyx jubatus*)

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Lifetime semen production data provide valuable insight into a species' natural history and biology as well as information about the potential fertility of males at various life stages. An understanding of the ages of sexual maturity, peak sperm production, and gonadal senescence will contribute to the design of species management plans for captive, free-ranging, and reintroduced populations of exotic animals. To describe these life stages in the cheetah, semen was collected biweekly for 13 years from a solitary male beginning at 3 years of age. The 338 ejaculates were obtained noninvasively by artificial vagina. Ejaculate volume and sperm motility score, concentration, and normal morphology were recorded. A sperm quality index incorporating all five semen parameters was calculated to facilitate ejaculate comparisons. Polynomial regression analysis revealed a significant effect of age on volume, which increased throughout the animal's lifetime except for a reduction between ages 10 and 12. Concentration was also significantly affected by age and increased from the age of 3 to the age of 10, then decreased. The sperm quality index revealed a significant effect of age as it increased from age 3 to age 8, then declined as the male grew older. The cheetah did not reach peak semen production until age 8 and continued to produce good quality semen for several more years. These data were somewhat unexpected, given the average cheetah life expectancy of approximately 7 years. Zoo Biol 20:359–366, 2001.

Key words: gonadal senescence; sexual maturity; artificial vagina

INTRODUCTION

Breeding and husbandry plans for captive exotic specimens occasionally require delayed reproduction, long-term separation of sexes, or removal of animals from breeding groups before reproductive senescence. These plans are often influenced by assumptions about the potential fertility of an animal before and after the

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years considered prime breeding age. An understanding of the species' ability to produce viable sperm over time may enhance management of captive males. However, the reproductive capacity of males throughout life has been examined in very few species other than humans. Domestic and laboratory animals are normally culled at the first sign of declining fecundity, and lifetime semen production data for exotic animals have not been reported.

Because most wildlife species are intractable, even in captivity, semen collection and evaluation requires immobilization and electroejaculation. The risks inherent in tranquilization or anesthesia limit the frequency with which collections can be performed. However, occasional or sporadic semen collection does not yield information reflective of sperm production potential because sperm quality declines over time in the epididymis [Cuasnicu and Bedford, 1989; Calvo et al., 1997; Noirault and Brillard, 1999]. Therefore, a noninvasive method of semen collection must be employed to compile a meaningful series of ejaculates that will describe a male's reproductive capacity.

To examine the effect of age and frequent collection on sperm production in cheetahs, data in this article represent the serial evaluation of one male throughout his adult life.

MATERIALS

Semen Donor

As the single survivor in a litter of two, this male cheetah (SB #291) was rejected by his dam. He was hand-reared for use in the education program at the San Diego Wild Animal Park. At 2 years of age, this male began to demonstrate sexual interest in enclosure enrichment items (tires, cardboard boxes) which eventually resulted in mounting and ejaculation. As this time, the male was transferred to a 50′ × 85′ outdoor enclosure within auditory, visual, and olfactory proximity to a variety of male and female conspecifics. He was fed a horsemeat-based diet 5–6 days per week with a weekly enrichment food item such as a chicken. The cheetah experienced no significant health problems until shortly before his death at 15.5 years of age. The male did not exhibit appropriate behavior in the presence of estrous female cheetahs and was therefore never introduced for breeding.

Semen Collection and Evaluation

At 2.8 years of age, the cheetah was trained to service an artificial vagina. No chemical or physical restraint was used, and a positive food reward regimen resulted in rapid, nonstressful semen collection. Ejaculates were collected until three months before the animal's death.

Ejaculates were extended immediately in TEST-Y buffer (1:1 vol:vol) and were allowed to slowly equilibrate to room temperature before evaluation for the following parameters: volume (V, in mL), concentration by hemacytometer count (C, × 10^6/mL), motility (M), speed of progression (SOP, based on a scale of 1–5, 5 being fastest), and normal morphology (%N). The number of motile sperm per ejaculate represented the product of the motility, concentration, and volume. Functional motility was expressed as a motility score ($MS = M \times SOP^2$). To facilitate comparisons of sperm quality between years, a sperm quality index (SQI) was calculated as $V \times C \times MS \times \%N/1{,}000$.

Statistical Analysis

Reported values represent the yearly mean for each measured parameter. All statistical analyses were performed using Statview® (Abacus Concepts, Berkeley, CA). The effect of semen collection interval on each measured parameter was expressed as a correlation. Because age did not affect semen parameters in a linear fashion, polynomial regression was used to generate best-fit curves.

RESULTS

Semen Collection and Evaluation

The number of ejaculates evaluated per year of age is shown in Figure 1 and is the same for each subsequent figure. Semen was collected two to four times a month for 13 years, yielding a total of 338 analyzable ejaculates. The collection interval ranged from 1.21 to 2.48 weeks, with a mean interval of 1.86 weeks. Twenty-six partial, temperature-shocked, or lubricant-contaminated ejaculates were excluded from the data set. Partial ejaculates were more frequent at ages 3 and 4 because of the young cheetah's tendency to be easily distracted by peripheral activity during the collection procedure. There were no significant correlations between collection interval and volume (r^2 = 0.294, P = 0.36), motility score (r^2 = 0.359, P = 0.26), concentration (r^2 = 0.006, P = 0.98), total motile sperm per ejaculate (r^2 = 0.460, P = 0.14), percent normal morphology (r^2 = 0.485, P = 0.11), or sperm quality index (r^2 = 0.431, P = 0.17).

Ejaculate volume gradually increased as the cheetah aged, beginning with a mean of 0.604 ml at 3 years (Fig. 1). A decline in volume was observed at ages 11 and 12, followed by an increase to the peak mean value of 2.26 mL at age 14. There was a significant effect of age (P = 0.0072) on semen volume as it increased, then decreased with advancing age.

The total motile sperm per ejaculate was significantly affected by age (P = 0.002, Fig. 2). A slow increase from age 3 to 7 was followed by an abrupt rise to three years of high numbers of sperm peaking at age 8 at 4,033 $\times$ 10^6, then steadily declining to the lowest number (168 $\times$ 10^6) at age 15.

The effect of age on motility score was not significant (P = 0.0717), probably because of high values at ages 12 and 13 (Fig. 3). However, there was a gradual increase from a low mean of 225 at age 4 to a peak of 700 and 800 at ages 12 and 13, respectively. By ages 14 and 15, motility scores had declined to levels approximating those seen at age 4.

Sperm concentration varied significantly (P = 0.0063) with age, generally increasing as the animal aged and reaching a peak of 33.1 $\times$ 10^6/ml at age 9, before beginning a steady decline to age 15 (Fig. 4).

Similarly to other cheetahs [Lindburg et al., 1993], this male consistently exhibited a low percentage of morphologically normal sperm. The lowest means of 24% at age 4 and 23% at age 15 were separated by two peaks of 49% normal at ages 8 and 13. The yearly mean percentage of normal sperm never exceeded 50% (Fig. 5). Although there was a tendency for the percent normal to increase to midlife and then decline, the changes over time were not significant (P = 0.0907).

With the exception of a decrease at age 4 and a secondary peak during year 13, the SQI increased from age 3 to age 8, then declined during the remaining years of the animal's life (P = 0.0045, Fig. 6). This pattern followed those seen for the measured parameters comprising the sperm quality index.

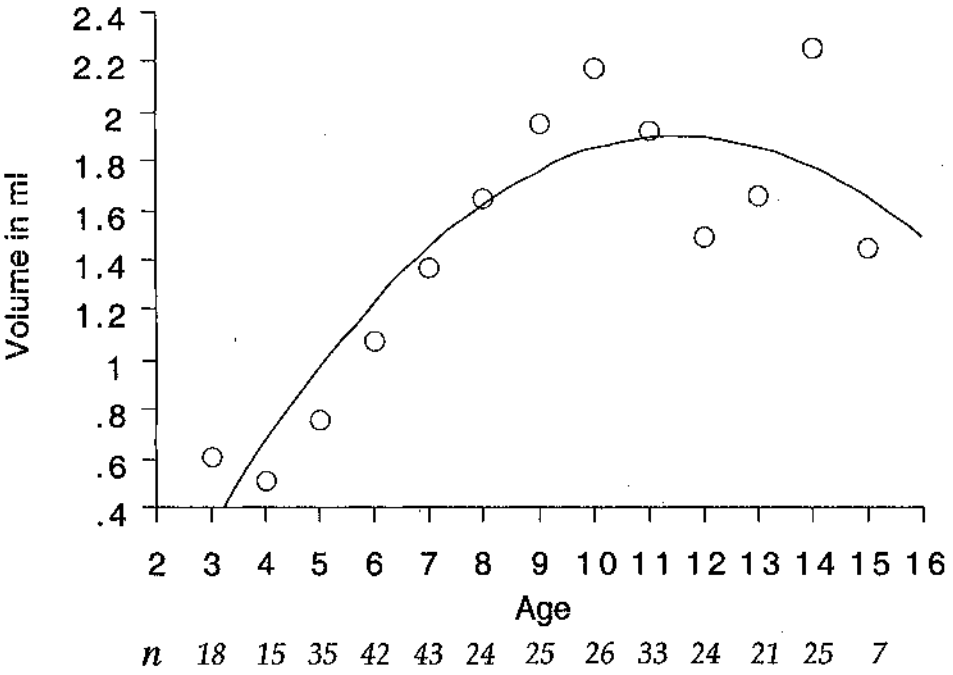

Fig. 1. Regression of ejaculate volume on age.

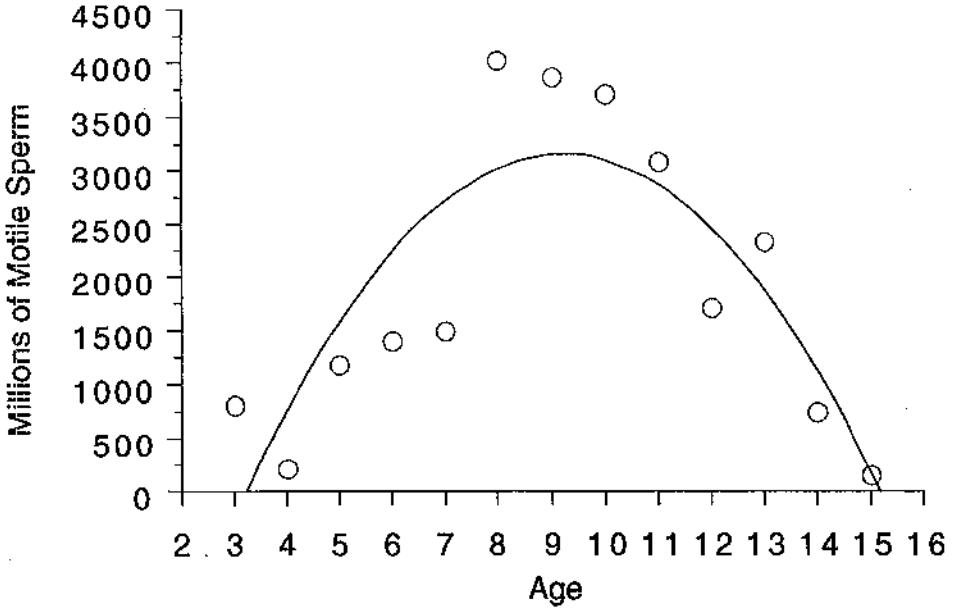

Fig. 2. Regression of total motile sperm per ejaculate on age.

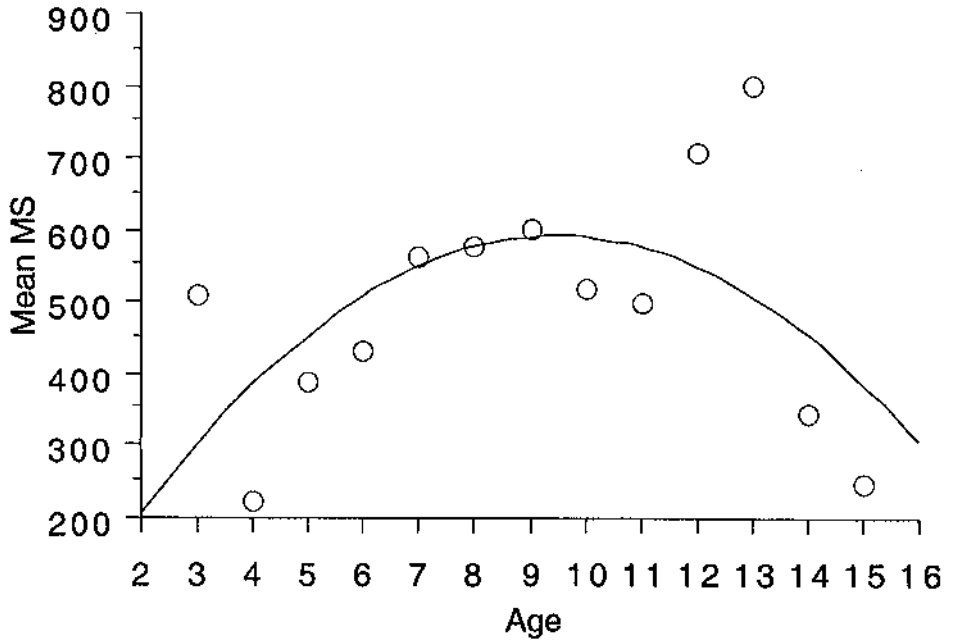

Fig. 3. Regression of motility score on age.

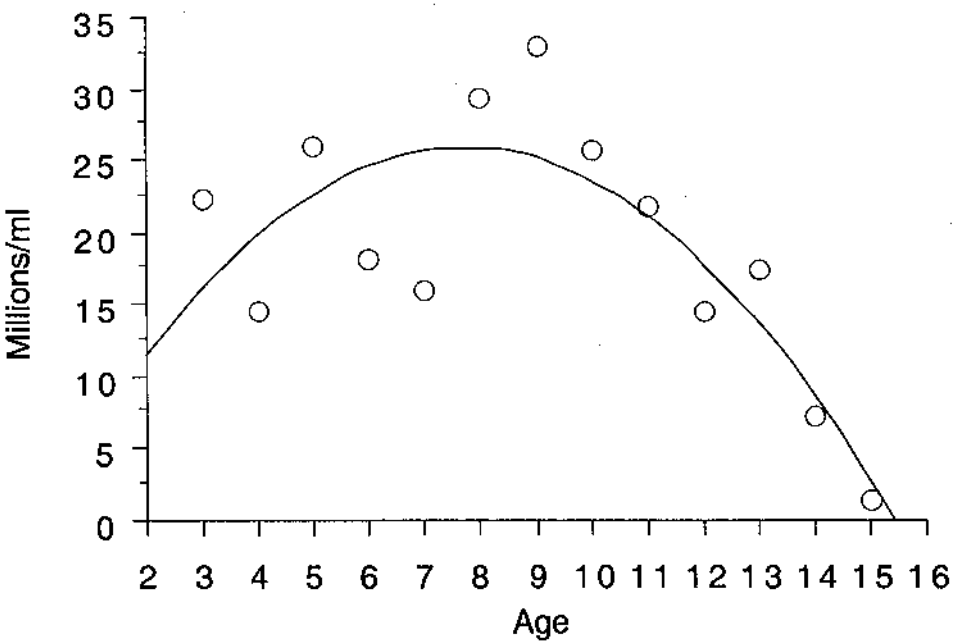

Fig. 4. Regression of sperm concentration on age.

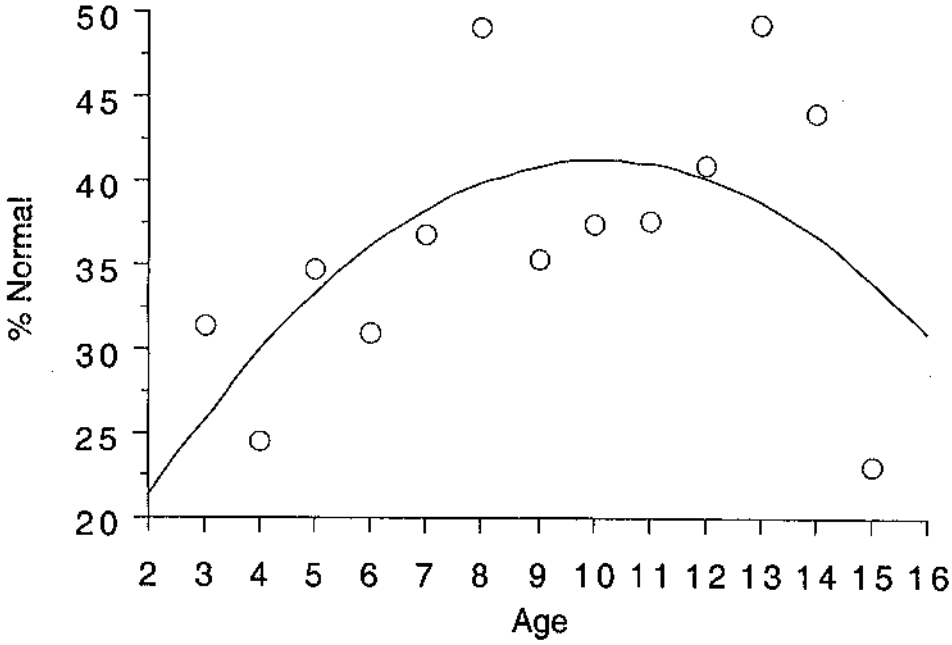

Fig. 5. Regression of percent normal sperm on age.

DISCUSSION

Sexual maturation and reproductive senescence in female mammals are recognized by the initiation and cessation of estrous cyclicity. These landmarks of reproductive function are not as easily discerned in the male, making the timing of social manipulations and pairings more difficult. Frequent semen collection may be the only noninvasive method of revealing these landmarks if the male is not consistently allowed access to fertile females. The single male cheetah in this study contributed an average of 26 semen samples per year for 13 years, a frequency that allowed the accurate monitoring of sperm production from early sexual maturity to extreme old age.

In the past, the cheetah's low reproductive rate in captivity has been linked to poor semen quality [Wildt et al., 1983; O'Brien et al., 1985]. However, most of the data contributing to these reports were compiled from one or two semen analyses per male without reference to the sexual activity or fertility of the animals. In subsequent studies, no differences were found between spermiograms of captive and free-ranging cheetahs [Wildt et al., 1987], essentially uncoupling the link between sperm quality and reproductive failure. More recently, it has been shown that cheetah fertility is not strongly correlated with semen parameters, because proven males consistently produce semen inferior to that reported for fertile males of other species [Lindburg et al., 1993]. In addition, improved husbandry practices have resulted in the establishment of several successful breeding groups in North America with no concomitant improvement in semen quality.

The data from this study indicated that although the cheetah's semen quality was consistently poor in comparison to most other mammal species, the effect of age on sperm production was profound. In general, semen quality increased to age 8–9,

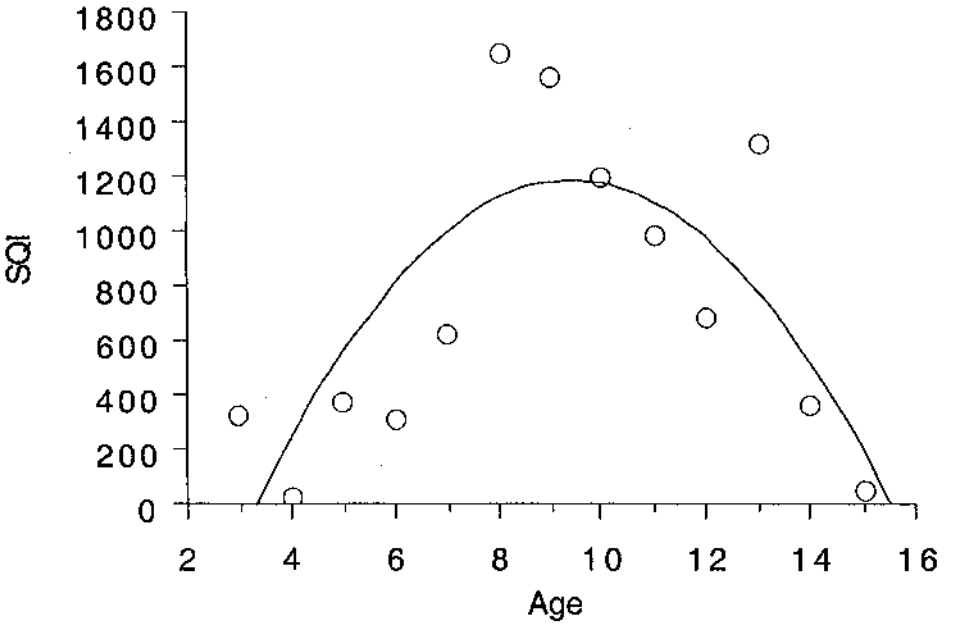

Fig. 6. Regression of sperm quality index on age.

then decreased. Notable exceptions were volume, which did not decline appreciably in old age, and concentration, which was similar during prepeak years but declined precipitously after age 9. Furthermore, although motility did not significantly change with time, the total motile sperm per ejaculate (the product of volume, concentration, and motility) was the most useful single parameter in demonstrating the effect of age on semen quality.

The causes of the decrease in SQI from age 3 to age 4 and the increase at age 13 are unknown. The low mean SQI at age 4 reflected reductions from age 3 in all four measured parameters (volume, motility score, concentration, and percent normal morphology), indicating an environmental or physiological perturbation too subtle for human observance. A single ejaculate analyzed during this temporary decrease in semen quality could result in the misdiagnosis of the animal's potential fertility. Other than these apparent divergences from the trend, the male's semen quality increased annually until age 8, and then began to decline steadily. The attainment of peak sperm production at 8 and 9 years of age was unexpected because captive cheetahs experience a short life expectancy with only 16% of the population reaching or exceeding 11 years of age (L. Bingaman-Lackey, Cheetah Regional Studbook Keeper, personal communication, 2001). Poor quality at age 15 probably reflected the male's deteriorating health and the normal degeneration of testicular function associated with aging.

Although these data were generated from a single male cheetah, they may give managers a better understanding of the time course of potential fertility in this species. For example, mating competence may be attained at 2 to 3 years of age, before the production of adequate numbers of normal sperm. Thus, pairing young males with reproductive females would probably be ineffectual. In addition, cessation of active breeding may not signal testicular senescence, and viable sperm may be col-

lected from aged males. Using the technique of artificial vagina semen collection, frequent sampling of other males will strengthen the database and create a reliable profile of reproductive potential in cheetahs.

The hand-raised animal in this study was not paired for breeding; therefore, the effects of semen collection and age on libido could not be assessed. However, a second, mother-raised male was conditioned for artificial vagina semen collection, which did not interfere with his willingness or ability to breed naturally and produce cubs [Durrant et al., 1997].

The tractable nature of cheetahs allows the opportunity for noninvasive semen collection for germplasm banking and artificial reproductive technology. Perhaps the most important result of this long-term study was the preservation of sperm from this genetically valuable male. Although he was not behaviorally capable of natural breeding, the Cheetah Species Survival Plan's Studbook and Master Plan recommended the infusion of his genes into the captive population. The number of stored ejaculates will allow the insemination of multiple females over many generations.

CONCLUSION

1. Peak semen production in a cheetah was attained at age 8, followed by a gradual decline interrupted by an unexplained increase at age 13.

2. By age 15, semen quality had returned to values seen during the early years of sexual maturity.

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