REPRODUCTIVE TRAITS INCLUDING SEASONAL OBSERVATIONS ON SEMEN QUALITY AND SERUM HORMONE CONCENTRATIONS IN THE DORCAS GAZELLE

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ABSTRACT

Seminal traits, testicular volume, body weight and serum testosterone levels were determined monthly for one year in 3 adult male Dorcas gazelles (Gazella dorcas). Serial blood samples were evaluated for cortisol and testosterone concentrations to determine the acute effect of electroejaculation, and the longevity of spermatozoal viability in vitro also was assessed. High percentages of progressively motile, normal spermatozoa were collected from all males throughout the year. Positive correlations were found between spermatozoal concentrations, testicular volume and body weight; however, no relationship existed between serum testosterone and either testes volume or seminal quality. No evidence of seasonality was observed in either seminal or hormonal traits. Serum cortisol and testosterone concentrations were not significantly different between anesthetized-electroejaculated gazelles and the same males subjected to anesthesia only. Spermatozoa longevity in vitro was prolonged by the addition of tissue culture solution to raw semen and further improved when seminal plasma was removed following centrifugation.

This study 1) provides a data base for a little-studied nondomestic species, the Dorcas gazelle; 2) indicates that seminal traits and serum testosterone concentrations are not influenced by season; 3) demonstrates that electroejaculation is an effective technique for repeatedly collecting semen samples without acutely or chronically influencing gonadal or adrenal function.

INTRODUCTION

Established artificial breeding techniques used in domestic animals could enhance captive propagation of selected zoological species.

However, efforts have been hindered because of the difficulty in obtaining non-domestic species for research purposes and the general lack of baseline reproductive-endocrine data which exists for most zoological specimens. Establishing reproductive norms is a prerequisite to the eventual practical application of artificial breeding of nondomestic species.

The present study examines seminal traits and serum hormonal concentrations in a medium-sized antelope, the Dorcas gazelle (Gazella dorcas). Native to North Africa and the Middle East, wild populations have declined sharply since 1960 as a result of over-hunting and habitat deterioration(1). The Dorcas gazelle breeds relatively well in captivity and its size is sufficiently small to permit manual restraint for blood sampling and anesthetic administration. This species was chosen for study because 1) no information exists on gonadal-endocrine relationships; 2) as a ruminant, artificial breeding techniques and estrous synchronization used successfully in domestic cattle and sheep could eventually have ready application; and 3) the Dorcas gazelle is not currently considered endangered and therefore could serve as an appropriate model for determining the influence of electroejaculation and handling a non-domestic animal over a prolonged interval.

The primary objective of this investigation was to determine ejaculate traits, testicular volume, body weight and serum testosterone concentrations in the male Dorcas gazelle during a 12-month period. Secondary objectives included evaluating 1) the stress-induced response of the electroejaculation procedure; and 2) the longevity of spermatozoal viability in vitro.

MATERIALS AND METHODS

Animals and anesthesia-Three adult male Dorcas gazelles were maintained in an outdoor enclosure with shelter at the National Zoological Park in Washington, D.C. (latitude 38°51'N and longitude 77°02'W). During the year of the study (1981), the animals were subjected to normal seasonal variation in climate. The ranges in daylight hours and outdoor ambient temperature for each season were Winter (Dec.-Feb.), 9.5 to 12.0 hours, -3.9 to 13.9°C; Spring (Mar.-May), 12.0 to 15.0 hours, 3.3 to 30.6°C; Summer (June-Aug), 12.0 to 15.0 hours, 17.2 to 31.1°C; Fall (Sept.-Nov.), 9.5 to 12.0 hours, 0.0 to 26.1°C.

Each male was anesthetized for electroejaculation at approximately the same time each month for one year. A total dose of 3 mg xylazine (a), injected intramuscularly, followed in 20 minutes with 150 mg ketamine hydrochloride (b) intramuscularly, was used. The time interval from xylazine administration to a surgical plane of anesthesia was 30-40 minutes. Body weights were obtained and laboratory calipers were used to measure the dimensions of each testis (length X width). The latter

(a) Rompun, Haver-Lockhart, Shawnee, KS.
(b) Ketaset, Bristol Laboratories, Syracuse, NY.

measurements were used to calculate total testes volume/animal using the conversion formula for a prolate spheroid ($V=4/3\ \pi\ ab^2$, where a is one-half the length and b is one-half the width)(2).

Electroejaculation-Following induction of a surgical plane of anesthesia, electroejaculation was initiated using a previously described procedure(3). In brief, a Teflon probe (diameter, 1.6 cm; length, 20 cm) containing 3 raised, longitudinal, stainless steel electrodes was lubricated and inserted into the rectum with the electrodes positioned ventrally. An AC, 60-Hz current was administered using an electrostimulator (c) and a regimented electroejaculation sequence consisting of a total of 80 stimuli given in 3 series. Series 1 and 2 consisted of 30 stimulations each, divided into 3 sets of 10 stimuli at 3, 4, 5 volts (series 1) and 4, 5, 6 volts (series 2). A total of 20 stimulations was administered in series 3, with 10 stimuli each at 5 and 6 volts. A rest period of 3-4 minutes was permitted between series. The electrical stimuli were given in a 3-sec-on and 3-sec-off pattern, with a continuous rise in voltage from 0 volts to the desired peak, then returning to 0. The ejaculate was collected in a warmed, plastic container.

Semen evaluation-Semen was evaluated immediately for ejaculate volume and microscopically assessed at 37°C for spermatozoal motility and status (speed of forward progressive motility). Spermatozoal status was a subjective evaluation based on a scale of 0 (lowest rating) to 5 (highest rating)(4). Spermatozoal concentration was determined using standard hemacytometer methods and calculated for spermatozoal count/ml of ejaculate and total spermatozoal count/ejaculate. In selected cases, ejaculate aliquots were 1) fixed in 1% glutaraldehyde for subsequent morphologic examinations; 2) used to determine duration of spermatozoal viability in vitro at 37°C. For morphologic evaluation, 300 spermatozoa/ejaculate were examined by phase-contrast microscopy (1000X) for pleimorphic defects in spermatozoal anatomy. Spermatozoal head, midpiece, and tail dimensions were also measured by phase-contrast microscopy (2500X). Viability evaluation of spermatozoa consisted of ascertaining percent motility and status at 15-minute intervals for up to 7 hours after one of 3 treatments. Freshly collected spermatozoa of each ejaculate was divided into 3 aliquots for handling: 1) untreated (raw aliquot); 2) diluted 1:1 (v/v) with a tissue culture solution (TCS) (d); 3) diluted 1:1 with TCS, centrifuged at 300 g for 10 minutes, the supernatant decanted and the spermatozoal pellet resuspended in TCS to a volume equal to twice the original aliquot volume.

Monthly testosterone evaluations-Following induction of anesthesia and prior to each electroejaculation, a blood sample (10 ml) was taken for radioimmunologic analysis of testosterone concentration. Following clotting and centrifugation, serum was collected and stored at -20°C until assayed. Testosterone was analyzed using a commercially

(c) P-T Electronics, College Station, TX.
(d) Tyrodes Tissue Culture Solution, Difco Laboratories, Detroit, MI.

available testosterone (^{125}I) radioimmunoassay kit(e). The antiserum, consisting of rabbit anti-testosterone-19-carboxymethylether-bovine serum albumin, binds 50-60% of testosterone (^{125}I) in the absence of non-radioactive testosterone. Inter- and intra-assay coefficients of variation were 7.9% (n=6) and 4.6% (n=8) and the lower limit of detection was 0.1 ng/ml.

Influence of electroejaculation on serum cortisol and testosterone-Serial blood samples were collected during randomly selected monthly procedures to determine the acute effect of electroejaculation on serum cortisol and testosterone. Samples were obtained immediately before the injection of each anesthetic, prior to initiation of electroejaculation, immediately after each of the 3 series and 15, 30 and 45 minutes after the last stimulation. For a comparative control, the 3 Dorcas gazelle males were anesthetized and serially bled at comparable intervals with no concomitant electrostimulation. Sera were analyzed for cortisol using a commercially available cortisol (^{125}I) radioimmunoassay kit (f), consisting of a combined first and second antibody containing rabbit cortisol antibody prereacted with an antiserum to rabbit gamma globulin. Inter- and intra-assay coefficients of variation were 5% (n=5) and 4% (n=8) and the minimum detectable assay sensitivity was 10 ng/ml.

Statistical Analyses-A computerized statistical program was used to analyze the data by individuals and by seasons for means, standard error of the mean (SEM) and analysis of variance (ANOVA). The Student-Newman-Keuls procedure was used to test significant ANOVA results for pairwise comparisons(5). Correlation coefficients (r values) were calculated between seminal traits, testosterone concentration, testicular volume and body weight. Hormonal responses were analyzed by graphically plotting profiles and comparing area under the curves using Student's t-test evaluations.

RESULTS

Semen was collected by electroejaculation from each Dorcas gazelle during each month of the year. Based on a total of 36 collections, the mean $\pm$ SEM total ejaculate volume was 1.8 $\pm$ 0.1 ml containing 381.4 $\pm$ 38.9 X10^6 spermatozoa/ml with a 85.7 $\pm$ 1.4% and 4.9 $\pm$ 0.1 motility and status rating, respectively. Mean spermatozoal head length, head width, midpiece length and tail length were 7.4, 3.7, 11.1 and 48.0 microns, respectively. A high percentage of morphologically normal spermatozoa was present in the ejaculate of each male (mean range, 88.1-90.7). An average of 11% of all spermatozoa exhibited morphologic defects including bent flagella (5.5%), proximal cytoplasmic droplets (2.5%), distal cytoplasmic droplets (1%), bent midpieces (1%) and tightly coiled flagella (1%).

(e) Radioassay Systems Laboratories, Inc., Carson, CA.
(f) New England Nuclear, North Billerica, MA.

Ejaculate characteristics varied among gazelles stimulated with the same number and intensity of electroejaculation stimuli (Table 1). Male 2 produced significantly greater ($P<0.05$) spermatozoal concentrations compared to the other gazelles. Although mean ejaculate volume was less in male 1 than in male 2 or 3, mean percent spermatozoal motility and status ratings were high in all males and did not differ significantly ($P>0.05$). Testicular volume and body weight varied ($P<0.01$) among individuals with male 2 consistently having the largest testes volume and maximum weight. In contrast, yearly serum testosterone averages did not vary among males. In general, ejaculate, hormonal and morphometric traits were not significantly correlated. However, total testes volume appeared related to body weight ($r=0.7$), spermatozoal count/ml of ejaculate ($r=0.6$) and spermatozoal count/ejaculate ($r=0.5$) but not serum concentration of testosterone ($r=-0.2$).

Ejaculate volume, percent spermatozoal motility, spermatozoal status, testicular volume and serum testosterone were not statistically different for the year, showing no evidence of being seasonally influenced (Table 2). Mean body weight was significantly increased ($P<0.05$) in the summer and fall in comparison to spring and winter values. Although mean spermatozoal concentrations (count/ml and total count) tended to be lowest in the winter and greatest in the spring, no particular season was significantly ($P>0.05$) different from another. Examination of individual profiles over time illustrate the randomness between months with no clear indication of seasonal influence (Fig. 1).

Males 1, 2 and 3 were subjected to 3, 1 and 3 periods, respectively, of serial bleeding before, during and after electroejaculation (Fig. 2). To serve as controls, males 1, 2 and 3 also underwent 2, 1 and 2 periods, respectively, of anesthesia and serial bleeding with no electrostimulation (Fig. 2). Mean serum cortisol concentrations in the latter group ranged from 24.8 ± 7.8 to 42.3 ± 11.7 ng/ml. Although cortisol did not vary significantly over time in control males, there was a tendency for concentrations of this hormone to be lowest during the midportion of the bleeding schedule or during deep anesthesia. In electroejaculated males, cortisol levels ranged from 38.3 ± 5.9 to 49.1 ± 6.1 ng/ml, the latter value measured immediately following the third series of electrical stimuli. Although the tendency existed for cortisol concentrations to be greater during electrostimulation, neither serum levels at any given time during serial bleeding nor the areas under the hormonal profiles (electroejaculation, 26 ± 3 cm^2 vs. controls, 23 ± 4 cm^2) were different ($P>0.05$) between groups. Little variation in ranges of mean serum testosterone was detected in the control (0.7 ± 0.2 to 1.3 ± 0.4 ng/ml) and electroejaculated gazelles (1.1 ± 0.3 to 1.9 ± 0.4 ng/ml) (Fig. 2). Testosterone tended to be greater following electroejaculation; however, concentrations were not significantly ($P>0.05$) different from prestimulation or control concentrations. No significant ($P>0.05$) influence or correlation ($r=0.2$) existed between cortisol profiles and testosterone concentration.

A total of 6 ejaculates was examined for spermatozoal longevity. Spermatozoal viability *in vitro* was affected by various handling methods used (Fig. 3). Adding tissue culture solution (TCS) to the raw

Table 1.--Ejaculate, Serum Testosterone and Morphometric Traits in Dorcas Gazelles

	Male 1	Male 2	Male 3
Ejaculate volume (ml)	1.4 ± 0.2[a] (0.5-2.5)[b]	1.9 ± 0.2 (1.0-2.7)	2.2 ± 0.3 (0.6-3.8)
Spermatozoa count per ml ($X10^6$)	293.8 ± 37.7 (105-510)	542.1 ± 90.9 (175-1064)	308.3 ± 0.3 (114-523)
Total spermatozoa count per ejaculate ($X10^6$)	434.6 ± 65.2 (63-714)	1048.8 ± 219.2 (262-2600)	644.6 ± 103.7 (129-1432)
Spermatozoa motility (%)	89.2 ± 1.4 (80-95)	86.7 ± 1.9 (70-95)	81.3 ± 3.2 (60-90)
Spermatozoa status	4.9 ± 0.1 (4-5)	5.0 ± 0.0	4.9 ± 0.1 (4-5)
Testicular volume (cm^3)	24.5 ± 0.9 (20.5-28.5)	37.5 ± 1.9 (28.2-47.9)	28.5 ± 1.1 (25.4-39.1)
Body weight (kg)	13.7 ± 0.2 (12.7-15.5)	16.0 ± 0.5 (12.3-17.7)	14.4 ± 0.2 (13.2-15.5)
Testosterone (ng/ml)	1.5 ± 0.7 (0.3-7.8)	1.0 ± 0.1 (0.3-1.7)	1.2 ± 0.3 (0.2-3.9)

[a] Values are means ± SEM.
[b] Numbers in parentheses are ranges.

Table 2.--Relationship of Season to Ejaculate, Hormone and Morphometric Traits in the Dorcas Gazelle

	WINTER (Dec-Feb)	SPRING (Mar-May)	SUMMER (June-Aug)	FALL (Sept-Nov)
Ejaculate volume (ml)	1.7 ± 0.30[a] (0.6-3.5)[b]	1.9 ± 0.2 (1.0-2.9)	1.6 ± 0.2 (0.5-2.7)	2.1 ± 0.3 (1.0-3.8)
Spermatozoal count per ml ($X10^6$)	235.9 ± 45.9 (105-549)	515.4 ± 100.7 (225-1040)	338.9 ± 41.3 (151-523)	435.3 ± 84.0 (179-1064)
Total spermatozoal count per ejaculate ($X10^6$)	391.4 ± 81.2 (63-823)	1016.1 ± 239.4 (234-2600)	562.6 ± 96.5 (75-1088)	867.1 ± 211.4 (383-2340)
Spermatozoal motility (%)	82.8 ± 2.5 (70-90)	83.3 ± 4.5 (60-95)	89.4 ± 1.5 (80-95)	87.2 ± 1.2 (80-90)
Spermatozoal Status	4.9 ± 0.1 (4-5)	4.9 ± 0.1 (4-5)	5.0 ± 0.0	5.0 ± 0.0
Testicular volume (cm^3)	28.1 ± 2.2 (20.5-39.1)	28.4 ± 2.3 (20.6-41.0)	31.6 ± 1.9 (26.8-43.9)	32.5 ± 3.2 (23.8-47.9)
Body weight (kg)	14.0 ± 0.3 (13.0-15.9)	14.0 ± 0.5 (12.3-16.8)	15.5 ± 0.5 (13.1-17.7)	15.4 ± 0.4 (13.6-17.7)
Testosterone (ng/ml)	1.6 ± 0.8 (0.2-7.8)	1.2 ± 0.4 (0.3-3.9)	0.8 ± 0.2 (0.3-1.7)	1.3 ± 0.5 (0.2-4.8)

[a] Values are means ±SEM.
[b] Numbers in parenthesis are ranges.

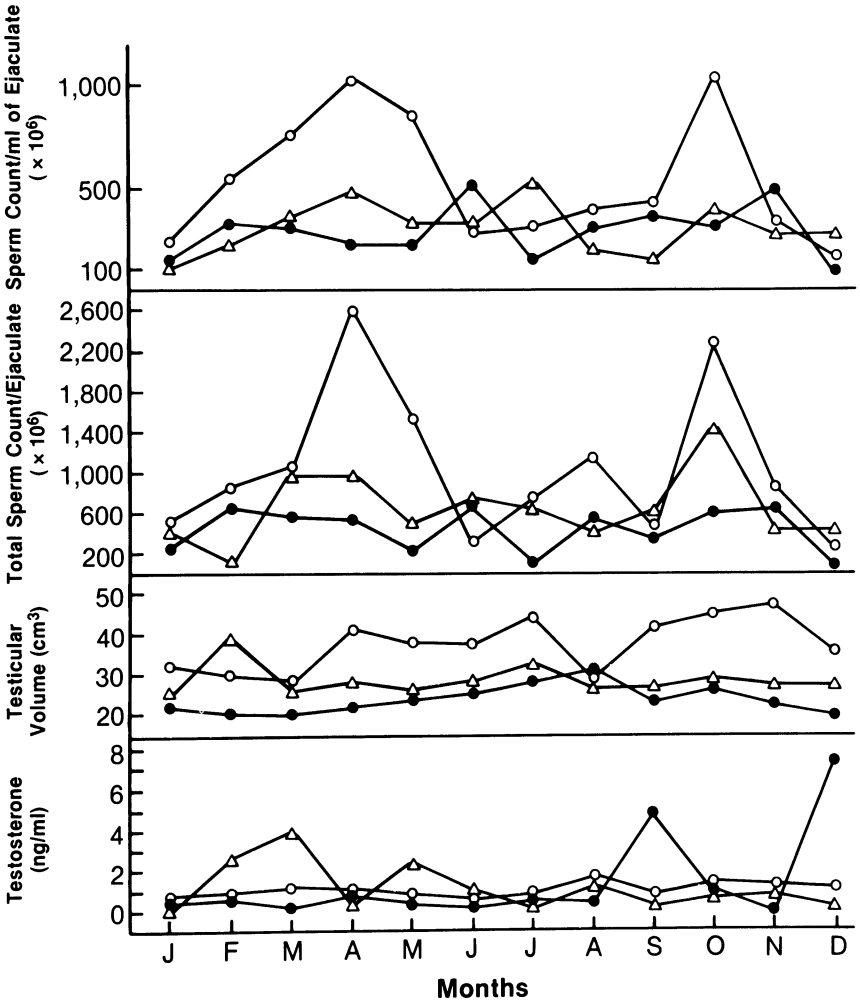

Fig. 1. Monthly spermatozoal concentrations, testicular volume, and serum testosterone concentration in Dorcas gazelles; male 1●—●, male 2○—○, male 3△—△.

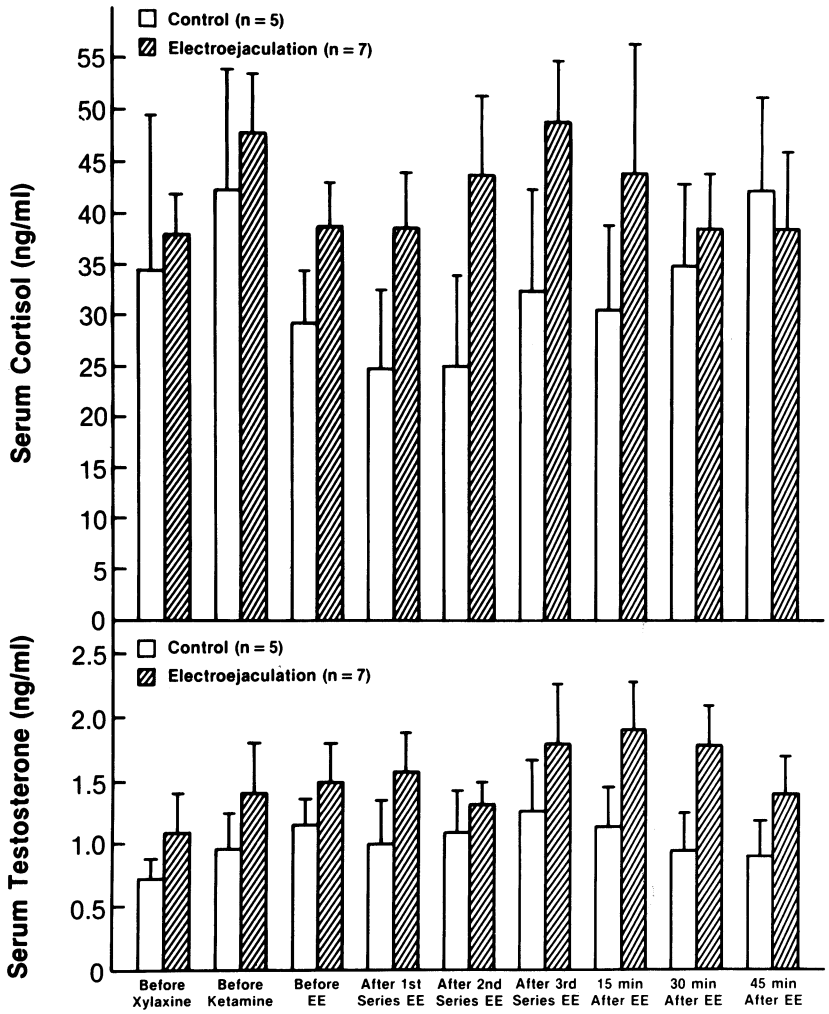

Fig. 2. Serum cortisol and testosterone concentrations before, during, and after electroejaculation (EE).

semen prolonged spermatozoal motility by 38%, increasing mean viability from 240 minutes (raw semen) to 330 minutes (raw and TCS). Furthermore, a substantial improvement ($P<0.01$) in duration of motility was detected when the seminal plasma was removed by centrifugation and the spermatozoal pellet resuspended in TCS. As illustrated in Fig. 3, mean spermatozoal motility was still 55% when samples were examined 420 minutes post collection. Overall, spermatozoal motility and status declined consistently as a function of time regardless of the handling method.

DISCUSSION

The chronic effects of handling, anesthetizing and electro-ejaculating a non-domestic species have not been studied previously. The present study demonstrated that electroejaculation was an effective and reliable procedure for collecting semen from the gazelle and, most importantly, that repeated episodes had no detectable influence on long-term ejaculate quality, gonadal function or general animal health. It also appeared that electroejaculation used repeatedly over time could be employed to distinguish individuals possessing the greatest reproductive potential. In our study using a limited number of animals and a regimented electrostimulation sequence, males tended to fall into a hierarchy, particularly in terms of the spermatozoal concentration traits.

Positive correlations were detected between spermatozoal concentration, testicular volume and body weight in the Dorcas gazelles. This observation was in agreement with similar findings in bulls in which scrotal circumference and seminal production and quality are highly correlated and testes size is effectively used to evaluate spermatogenic capacity and fertility potential.(6) Correlations between gonadal size and body weight also are reported in bulls(7). The present study, however, demonstrated no relationship between serum testosterone and either testes volume or semen quality in the Dorcas gazelle. Similarly, serum testosterone values are an inadequate index of fertility in domestic bovids(8). It can be concluded that blood testosterone evaluations in gazelles have little value in predicting fertility.

The quality of spermatozoal activity and proportion of morphologically normal sperm were not affected by the electroejaculation procedure; high percentages of progressively motile, normal spermatozoa were collected from all males throughout the year. Similarly, studies in domestic ungulates have demonstrated that electroejaculation has no deleterious influence on spermatozoal quality(9). Ejaculate volume may be greater and spermatozoal concentration less in electroejaculates compared to ejaculates obtained using an artificial vagina, primarily because the former procedure causes increased stimulation of accessory gland secretion(10,11). The gross appearance and dimensions of the Dorcas gazelle spermatozoa were similar to that described for a close taxonomic relative, the Speke's gazelle (*Gazelle spekei*)(12) but were slightly smaller than male gametes from domestic ungulates such as cattle (13) and sheep (14).

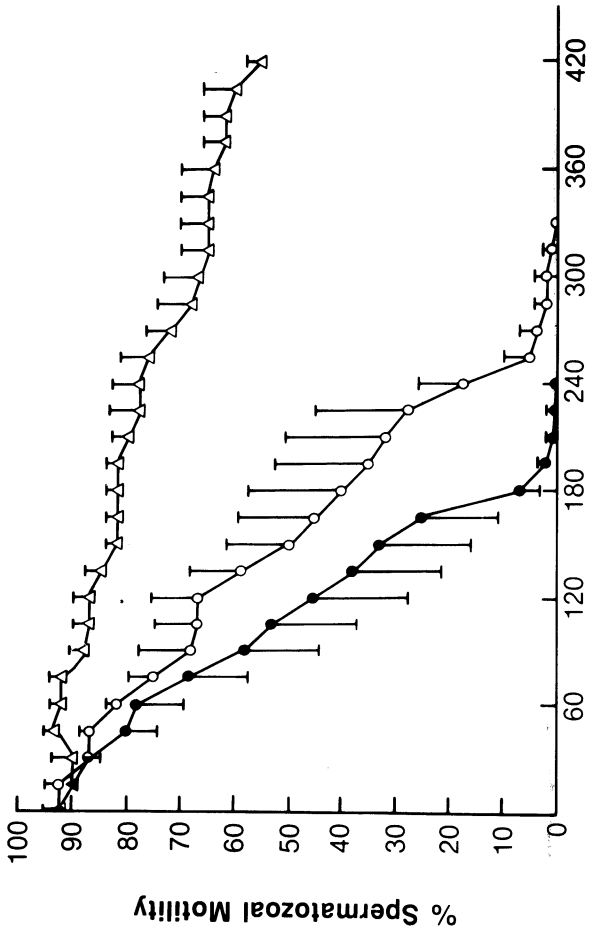

Fig. 3. Longevity of spermatozoal motility in vitro of raw semen (●—●), raw semen + tissue culture solution (TCS)(○—○), and raw semen + TCS + centrifugation (△—△).

Considering semen durability, the viability of Dorcas gazelle spermatozoa was increased from 4 hours to 5.5 hours by simply diluting the ejaculate with tissue culture solution. Substantial further improvement in longevity was achieved by removing the seminal plasma after centrifugation and resuspending the spermatozoa in tissue culture solution. The centrifugation protocol used was the same as that reported previously in handling domestic bovid spermatozoa(15) and, as in the bull, did not adversely affect spermatozoal viability.

Electroejaculation had no influence on adrenal or gonadal hormonal secretion in the gazelle. Because serum cortisol and testosterone concentration were not significantly altered, it may be presumed that electroejaculation of the anesthetized gazelle is a minimal physiological stress. In contrast, in unanesthetized bulls, cortisol levels increase (6-fold) from 20 ng/ml to 125 ng/ml as a result of electroejaculation and remain significantly elevated for 2 hours(16). Decreased testosterone secretion (<2 ng/ml) also is detected in bulls for up to 6 hours following electrostimulation in comparison to concentrations before electroejaculation (2-6 ng/ml).

For many species existing in the wild, reproduction often is seasonal to ensure that offspring are produced at times coincident with maximizing survival. Dorcas gazelles are reported to be seasonal breeders in their natural habitat, although it is unknown whether periodic sexual inactivity is the result of suppressed reproductive function in the male or female or both sexes(17). The present study demonstrated no seasonal effects on ejaculate quality or serum hormone concentrations in captive-bred male Dorcas gazelles. Behavior and birth records from the National Zoological Park indicate that males of this species are sexually active throughout the year(18). Furthermore, reproductive function in other gazelle species maintained in captivity also apparently is not influenced by season since calves have been born throughout the year(19,20). These data imply and the present study confirms that the gazelle has adapted to its secure zoological environment by maintaining peak reproductive capacity throughout the year.

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