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NOTE

SEMEN CHARACTERISTICS, SEXUAL BEHAVIOR AND VARIATION OF SERUM TESTOSTERONE CONCENTRATIONS IN CASPIAN PONIES

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ABSTRACT

Semen characteristics, plasma testosterone concentrations, and sexual behavior of Caspian pony stallions were investigated. Semen was collected by using an artificial vagina, after teasing with a mare in standing estrus, and peripheral blood was sampled every 2 wk from 7 Caspian pony stallions throughout one year. Semen evaluation showed that semen volume, sperm concentration, motility and morphology, and testosterone concentrations varied among individuals. Testosterone concentrations were significantly greater during the first trimester of the breeding season.

KEY WORDS: Caspian pony, Libido, Semen, Testosterone.

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ارزیابی منی، رفتار جنسی و تغییرات تستو سترون در اسبچه هـــای ناحیه دریای خزر

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به ترتیب استادیار ســابق بخش علـوم درمانگـاهی، دانشـکده دامپزشـکی، دانشـگاه شـیراز (اکنون در بخش علوم درمانگاهی، دانشکده دامپزشـکی، دانشـگاه آزاد اسـلامی کـرج، کـرج، جمهوری اسلامی ایران) و استادیار سابق بخش علوم دامی، دانشکده کشـاورزی ، دانشـگاه شیراز، شیراز، جمهوری اسلامی ایران.

چکیده

آزمایش منی بویژه اگر با توجه به مشـاهدات و معاینـات بـالینی انجـام گـیرد بهـترین روش ارزیابی توان تولید مثل در حیوانات نر است. در این پژوهش، در طول یکسال، هفتـه ای یک بار از ۷ رأس سیلمی بالغ، نژاد اسبچه دریای خزر پس از روبه رو کردن آنها با مادیـان فحل و پرش، با استفاده از واژن مصنوعی منـی جمـع آوری شـد. هـر دو هفتـه یکبـار خـون سیاهرگی جانبی آنها به منظور اندازه گیری و بررسی تغییرات تستوسترون پلاسما تهیه شد. پـس از آزمـایش هـای معمـول، مشـخص گردیـد کـه حجـم منـی در هـر انـزال، تعـداد اسپرماتوزوئید های طبیعی، تحرک اسپرم و میزان تستوسترون خون در هر یک از حیوانـات مورد آزمایش تفاوت های قابل توجهی را نشان می دهد. میزان تستوسترون پلاسما در نیمـه اول و دوم (دوره های فعالیت تناسلی مادیان) به خصوص نیمه اول بطـور مشـخصی بیشـتر از سایر فصول سال بود.

INTRODUCTION

Examination of semen, if carried out with a clinical examination, is the most practical method for evaluation of reproductive soundness in male animals. Little attention has been given to stallion semen in the past; however, in recent years, a number of reports have appeared about semen characteristics, blood testosterone concentrations, and seasonal influences on stallion fertility (1, 3-6, 9, 10, 13-19). This paper presents data on

semen characteristics, plasma testosterone concentrations and the mating behavior of the Caspian stallions, the Iranian breed known to have odd chromosomal numbers and low fertility.

MATERIALS AND METHODS

Seven entire Caspian stallions, ranging in ages between 2 and 11 yr, randomely selected and purchased from the Caspian area were used in this study. The stallions were kept outdoors at the School of Veterinary Medicine of Shiraz Univeristy. The ration consisted of oat, alfalfa hay, and wheat hay throughout the study, except during the month of April when they had access to fresh grass. No pasture grazing was available. The names and ages of experimental stallions are presented in Table 1.

Table 1. Names and ages of the stallions used in the study.

Stallion	Age (yr)
Azin	9
Ahoo	3
Arsam	4
Zal	2
Shahin	2
Gord	3
Vandasb	11

After a mare in standing estrus was introduced to each stallion, the behavior, reaction time (from introduction of estrous mare to full erection and beginning of mounting), the time taken from first intromission into the artificial vagina (A.V.) till onset of tail flagging and ejaculation, and ejaculation period (first to last flagging of the tail) were observed and recorded. Semen was collected into a pony size A.V. Semen sample was immediately taken to the nearby laboratory. Semen was transferred into a graduated cylinder. The inner wall of the A.V. and the

collecting tube was washed thoroughly with an antiseptic solution, rinsed under tap water, dried, and prepared for the next collection.

Semen was collected regularly once a week starting from April and continuing through spring and summer until late October and early November, as long as mares in estrus were available.

Semen volume (gel-free, gel and total volume) and pH, and sperm motility, concentration, abnormalities, and sperm count per ejaculate were determined and recorded as described by Pickett and Back (13).

Blood was collected in heparinized tubes from the jugular veins at least every 2 wk for 12 mo. The plasma was separated immediately after collection and kept in a deep freeze until hormone assay. Testosterone was measured by radioimmunoassay (7), using [1, 2, 6, 7, -H]-testosterone (the Radiochemical Centre Ltd., Amersham, England).

RESULTS

The reaction time was quite variable. It was almost 3 times more than the period from first intromission into the A.V. and the onset of tail flagging and the period of ejaculation (54.50±58.83 seconds, reaction time comparing with 15.22±17.58, intromission to ejaculation and 15.98 ±13.29, ejaculation period). Mean and standard deviation for semen volume, sperm concentration and pH are presented in Table 2.

Two stallions (Arsam and Zal) always had abnormal behavior (vigorous and very little interest, respectively) with occasionally partial erection but never attempted to mount the estrous mare. They both had normal external genital tracts. It was not possible to collect semen from these stallions, therefore, they were excluded in that consideration.

Table 2 shows that individual variations in semen volume and sperm concentrations were great. No seasonal influence was found on the characteristics of the semen collected during various times of the year. From November onward when the breeding season of the Caspian mares was ended, semen collection was possible from 1 or 2 stallions, using a quiet mare which was not in estrus.

Generally, the total number of abnormal spermatozoa was quite high, mostly mid-piece, coiled or irregular in shape (Table 3). The mean number of sperm per ejaculate was 4.658×10^9, and in 62 samples examined, the sperm motility was 63.5±14.2 (mean ± SD) percent.

Table 2. Semen characteristics in weekly samples of 5 Caspian ponies.

	Semen volume (ml)				
	Gel-free	Gel	Total	Sperm concentration (millions ml^{-1})	pH
Mean	21.13	2.17	23.30	220.49	7.48
SD	17.11	3.82	19.79	169.72	0.26
No. of samples	65	65	65	67	67

Table 3. Percentage of various sperm abnormalities in Caspian horses based on 67 semen samples.

Sperm abnormality	%
Headless	2.16
Neck-separated (irregular)	0.41
Coiled midpiece	9.98
Reversed tail	0.83
Coiled tail	0.77
Proximal droplet	0.44
Distal droplet	0.30
Tail-less	2.81

Plasma testosterone concentrations were quite variable both between and within individuals. Mean testosterone values ranged between 85.22±53.03 pg ml^{-1} in October and 470.76±46.50 pg ml^{-1} in June. Table 4 shows mean concentration of the plasma testosterone in different mo, and Table 5 indicates this variation during 4 seasons.

The results of testosterone assay in Arsam and Zal which showed to be (clinically) impotent ranged between 39.4 pg ml^{-1} in November and 535.6 pg ml^{-1} during July for Arsam and, Zal had the minimum testosterone

concentration of 44.2 pg ml^{-1} in June and the maximum level of 346.4 pg ml^{-1} in November. Vandasb who was regularly used as teaser had the highest mean concentration of 421.0 pg ml^{-1}(range= 82.1 to 1246.3 pg ml^{-1}).

Table 4. Monthly concentration (pg ml^{-1}) of testosterone.

Month	Mean ± SD	No. of samples
April	298.35±275.20	2
May	277.69±229.28	8
June	470.76±465.16	8
July	193.55±173.10	7
August	169.06±171.05	7
September	187.51±105.56	8
October	85.22± 53.03	6
November	157.45±137.85	5
December	-	-
January	235.25±161.44	5
February	308.82±118.90	2
March	-	-

Table 5. Seasonal variation of plasma testosterone concentration (pg ml^{-1}).

Season	Mean±SD	No. of samples
Spring	358.3±331.6	15
Summer	214.4±208.9	25
Fall	118.0±102.1	11
Winter	256.3±145.0	7

DISCUSSION

Sexual behavior, particularly mating performance and the results of semen evaluation can be used as a practical method for evaluating the reproductive soundness of an individual as well as a group of stallions (4, 14, 20).

Our results show that there are some differences regarding semen characteristics of Caspian horses compared with other breeds. Lower semen volume (average, 23.3 ml) could be explained by the smaller size of the testicles in ponies (13, 14). On the other hand the mean concentration of sperm which will consequently affect the number of sperm per ejaculate was considerably low, as compared with other reports (5, 12, 18, 19).

Individual variations of the sperm concentration was great. Nonetheless, sperm motility of the semen in Caspian ponies appeared to be reasonably good and comparable with other studies (9, 18, 19). On the other hand, the percentage of abnormal sperm of the Caspian ponies found in this experiment is close to some of the previous reports on different breeds (2, 5, 10, 11).

The results of testosterone changes during different times of the year found in our study confirmed a previous report (1) of the maximum testosterone level during October, with the exception that in our study high testosterone concentration was found in June (Table 4).

It is interesting that the mean concentrations of plasma testosterone for all 5 stallions were low during October whereas the differences between stallions were great at other times of the year. It can be concluded that testosterone concentrations are high during spring and summer (Table 5) which agrees with the difference on sexual behavior of the stallions during breeding and non-breeding seasons. This has already been pointed out by Berndtson *et al.* (1).

The results of our study provide basic and general pieces of information on the reproductive parameters of the Caspian stallion, a breed which is supposed to have low reproductive capacity (8). Previous investigations indicated that the female of this breed has a comparatively low reproductive capacity (unpublished data). This could also be true for Caspian stallions. Two out of seven stallions were unable to mount the estrous mares and consequently had no ejaculations for analysis throughout the experiments.

Further detailed studies are required for a better judgment on reproductive capacity of the Caspian stallions.

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