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Monthly Evaluation of Testicular Biometry and Sexual Behavior With Respect to Seminal Aspects of Arabian Stallions Under Subtropical Conditions in Kuwait

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Abstract | Many factors, including the environmental temperature, photoperiod and the season of year have been shown to influence stallion reproductive performance. As latitudes greatly affect these factors, the current study aimed to, evaluate the influence of year months and seasons on testicular biometry, sexual behaviors and semen parameters of purebred Arabian stallions in Kuwait. Nine fertile stallions aged 4–22 years were used for assessment of testicle biometry, sexual behaviors and semen quality collected using artificial vagina throughout the year. Our results showed a clear monthly differences with higher semen quality ($P < 0.05$) especially in semen volume, sperm count, livability, and motility during June and July. Similarly, the length, width, height and volume of testes as well as the width of total scrotum were higher ($P < 0.05$) during August, July and September. Moreover, the number of mounts per ejaculation, reaction time, time to erection, ejaculation times and time to ejaculation were significantly lower during summer and spring seasons ($P < 0.05$). A positive correlation ($P < 0.05$) between sperm parameters and testicular parameters and negative correlation between the sperm parameters and sexual behaviors were recorded. These data suggest that, the testicular biometry and sexual behavior during summer and spring showed better characteristic than that during winter.

Keywords | Kuwait, Arabian stallions, Testicle biometry, Sexual behaviors;, Semen characters.

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INTRODUCTION

Photoperiod has been reported as the most important signal regulating the seasonal reproductive activity (Bronson, 2009). Horses, long-day seasonal breeders, exhibit annual cycles of breeding activity. Many physiological and ambient factors, as environmental temperature and length of photoperiod, affect reproductive activity of stallion. Seasonal changes in sperm production and testicular size (Clay et al., 1987), seminal pH and sex drive represented by number of mounts/ejaculate and reaction time have been recorded (Abou-Ahmed et al., 1993).

Horses show better reproductive performance during spring and summer, when the environmental temperature and daylight are very appropriate and good quality food is available, as these factors reduce reproductive activity in winter months (Gerlach and Aurich, 2000). In stallions, variations among seasons were found to greatly affect sexual behavior, volume of semen and sperm motility, which

were higher in summer and spring than winter (Suliman et al., 2020).

During a certain seasons of the year, stallions show some difference in their reproductive performance, as testicular size, reproductive behavior and sperm production (Gerlach and Aurich, 2000; Zervos et al., 2010). For stallion, the width of total scrotum (WTS) was measured as a representative index of testicular size (Faber and Roser, 2000; McCue, 2021). Stallion testicular weight was significantly correlated with spermatozoal output (Gebauer et al., 1974). Moreover, the width of each testicle has been reported as the best correlates with testicle weight (Najjar et al., 2012). Evaluation of the reproductive behavior is an essential part of during assessment of breeding potential or evaluation of reproductive problems in stallions (Rua et al., 2015). The sexual behavior of Arabian stallions during seasons of the year in Egypt has been evaluated and was the best during summer season under Egyptian environmental conditions (Zeidan et al., 2017). Although stallions with poor libido may have excellent semen quality, the fertility of those stallions may be reduced.

Based on the fact that latitudes greatly affects the photoperiods, this study aimed to evaluate the changes in sexual behaviors, testicular biometry and semen characteristics in Arabian purebred stallions during year months and different seasons under environmental conditions in Kuwait.

MATERIALS AND METHODS

Ethics statement

All of the experimental procedures and methods were conducted following the Ethics for Humane Treatment of Animal Use in Research Guidelines.

Data location

The study was conducted in Subhan, Kuwait (latitudes 28.45° - 30.05° north and longitudes 46.30° - 48.30° east and subtropical climate predominance). Average temperature, humidity, precipitation, sunshine and daylight is shown in (Table 1). Kuwait has a climate of desert type, which is characterized by a long dry hot summer and warm short winter sometimes rainy and hardly noticeable secondary seasons (spring and autumn). The year seasons in Kuwait are winter (from December 6 to February 15), spring (from February 16 to May 20), summer (from May 21 to November 4) and autumn (from November 5 to December 5).

Animals

Nine fertile stallions aged 4–22 years were housed in closed stables provided with open yard for exercise. They were fed on a balanced ration composed of hay, barley and wheat bran throughout the year in addition to green fodder during winter season and they got water *ad libitum*.

Testicular biometry

Testicle biometry was evaluated twice monthly (every 14 days) throughout one year (December 2019 to November 2020). Length (distance between cranial and caudal pole), width (distance between the lateral and the medial side), height (distance between the dorsal and the ventral surfaces) were recorded using digital caliper for each testis and testicular volume (width x height x length x 0.5333) was recorded according to (Love et al., 1991; Waddington et al., 2017). Thickness of scrotal skin (TS) was recorded using digital caliper and width of total scrotum (WTS; the width across both testes together) was recorded using plastic meter according to (Thompson et al., 1979; Clay et al., 1987).

Sexual behavior parameters

The sexual behaviors were recorded twice monthly throughout one year from December 2019 to November 2020 as presented by (Clay et al., 1987; Waddington et al., 2017). Sexual behavior parameters were as follows: Number of mounts/ejaculation, Reaction time (interval between introducing the mare and ejaculatory mount), Time to erection (interval between introducing the mare and full erection), Time to ejaculation (interval from erection to full emission of semen), and ejaculation time (interval from intromission to complete emission of semen).

Semen collection

Ejaculates were collected from all stallion, monthly throughout one year using a Missouri model artificial vagina (Minitüb comp.) pre-warmed up to 42 °C and lubricated with white vaseline. After collection immediately, the semen was transferred and examined in the laboratory.

Semen evaluation

The collected semen was assessed for total volume (ml) and gel free volume (ml) using graduated cylinder as previously described (Aurich, 2016). The sperm concentration x 10^6 / ml was estimated using SpermaCue® (semen analyzer, Minitube) as previously mentioned (Köhne et al., 2020) and the total sperm count x 10^9 /ejaculate (Con/ejac) was evaluated by multiplying sperm concentration by semen volume. The pH was determined with the waterproof pocket pH tester (HI98107, Hanna, USA). The sperm viability % was estimated using eosine-nigrosine stain, while sperm motility % and progressive motility % were assessed using conventional microscopy after dilution with an isotonic buffer and then examined on a phase contrast microscope (Janett et al., 2003; Waddington et al., 2017; Kandiel and El Khawagah, 2018).

Table 1: Average temperature, humidity, precipitation, sunshine and daylight during the period of data collection in Kuwait©

Months	Temperature (°C)	Humidity (%)	Precipitation (mm)	Sunshine (hours)	Daylight (Hours: minutes)
January	13.5	61	30	7.5	10:28
February	15.9	61	15	8	11:06
March	20.8	61	15	8	11:56
April	26.6	55	15	8.5	12:50
May	32.6	55	15	10	13:35
June	36.8	49	0	11	13:57
July	38.4	41	0	11.5	13:47
August	38.1	46	0	11.5	13:09
September	34.3	51	0	11	12:17
October	29.1	60	5	9.5	11:23
November	20.3	59	20	8	10:38
December	15.1	65	25	7.5	10:17

Table 2: Monthly changes in semen parameters of Arabian stallions

	TSV	GV	GFV	Con/ml	Con/ej	Liv %	Motil %	Psm %
December	36.56 ± 1.51^{ef}	4.67 ± 0.41^{cd}	31.89 ± 1.24^{ef}	294.44 ± 8.84^{a}	9.32 ± 0.22^{de}	69.67 ± 1.15^{d}	64.67 ± 0.94^{c}	59.89 ± 0.93^{e}
January	36.56 ± 1.51^{ef}	4.67 ± 0.41^{cd}	31.89 ± 1.24^{ef}	293.33 ± 10.54^{a}	9.26 ± 0.12^{de}	72.33 ± 1.17^{bcd}	64.67 ± 0.94	60.44 ± 1.07^{e}
February	35 ± 2.6^{f}	4.78 ± 0.46^{cd}	30.22 ± 2.53^{f}	287.78 ± 9.25^{a}	8.64 ± 0.7^{e}	72.67 ± 1.57^{bcd}	69.11 ± 1.03^{ab}	60.89 ± 0.99^{e}
March	43.78 ± 1.53^{bcd}	5.22 ± 0.4^{bcd}	38.56 ± 1.31^{bcd}	270 ± 16.07^{a}	10.32 ± 0.56^{bcd}	74.33 ± 1.33^{abc}	70.11 ± 1.24^{ab}	64.89 ± 1.2^{bcd}
April	48.22 ± 1.64^{abc}	6.11 ± 0.61^{abc}	42.11 ± 1.82^{abc}	271.11 ± 16.11^{a}	11.22 ± 0.34^{abc}	76.11 ± 1.43^{ab}	71.56 ± 1.32^{ab}	67.11 ± 1.42^{ab}
May	48.78 ± 2.36^{ab}	6 ± 0.53^{abc}	42.78 ± 2.3^{ab}	272.22 ± 14.32^{a}	11.55 ± 0.7^{ab}	75.67 ± 1.47^{abc}	71.22 ± 1.08^{ab}	65 ± 0.58^{bcd}
June	50.11 ± 2.06^{a}	6.78 ± 0.36^{a}	43.33 ± 2.04^{ab}	276.67 ± 13.12^{a}	11.89 ± 0.59^{ab}	77.22 ± 1.75^{a}	73.11 ± 1.7^{a}	66.56 ± 1.46^{abc}
July	52.67 ± 2^{a}	6.78 ± 0.36^{a}	45.89 ± 1.8^{a}	271.11 ± 14.57^{a}	12.3 ± 0.52^{a}	78.11 ± 1.82^{a}	73.11 ± 1.7^{a}	67.78 ± 2.18^{ab}
August	47.67 ± 2.46^{abc}	6.28 ± 0.52^{ab}	41.39 ± 2.02^{abc}	257.78 ± 16.31^{a}	10.53 ± 0.59^{bcd}	77.33 ± 1.12^{a}	73 ± 1.09^{a}	69.11 ± 0.93^{a}
September	42.22 ± 2.08^{cde}	5.22 ± 0.4^{bcd}	37 ± 1.74^{cde}	268.89 ± 14.67^{a}	9.89 ± 0.56^{cde}	74.33 ± 1.33^{abc}	69.44 ± 1.18^{ab}	62.67 ± 0.82^{de}
October	39.56 ± 2.02^{def}	4.78 ± 0.46^{cd}	34.78 ± 1.57^{def}	272.22 ± 13.41^{a}	9.42 ± 0.51^{de}	72.44 ± 0.97^{bcd}	67.44 ± 1.17^{bc}	61.44 ± 1.03^{de}
November	37.78 ± 1.7^{def}	4.22 ± 0.36^{d}	33.56 ± 1.43^{def}	281.11 ± 12.96^{a}	9.36 ± 0.42^{de}	71.44 ± 1.34^{cd}	68 ± 1.72^{bc}	63 ± 1.25^{cde}

TSV; Total semen volume, GV; Gel volume, GFV; Gel-free semen volume, Con/ml; Concentration/ml x 10^6, Con/ej; Concentration/ejaculate x 10^9, Liv; Livability, Motil; Motility, Psm; Progressive sperm motility

Statistical analysis

Data were analyzed using the SPSS software (IBM, SPSS Statistics, Version 22, USA). The data were expressed as mean ± standard error of means (SEM). One-way ANOVA was used to compare the effect of year months and four year seasons (spring, summer, autumn and winter). Duncan Multiple Range test was used to differentiate between significant means at $P < 0.05$.

Table 3: Testicular biometry of Arabian stallions along year seasons

Seasons	Length (cm)	Width (cm)	Height (cm)	TV (cm^3)	TS (mm)	WTS (cm)
Spring	9.67 ± 0.02[b]	6.99 ± 0.07[b]	5.66 ± 0.02[b]	397.0 ± 4.3[b]	9.2 ± 0.03[b]	13.51 ± 0.18[a]
Summer	9.96 ± 0.06[a]	7.33 ± 0.08[a]	5.81 ± 0.05[a]	419.0 ± 4.46[a]	8.94 ± 0.04[c]	13.87 ± 0.16[a]
Autumn	9.68 ± 0.03[b]	7.0 ± 0.07[b]	5.44 ± 0.03[c]	383.11 ± 5.47[c]	9.3 ± 0.04[b]	13.47 ± 0.11[a]
Winter	9.36 ± 0.02[c]	6.5 ± 0.05[c]	5.38 ± 0.03[c]	339.78 ± 3.84[d]	9.41 ± 0.03[a]	12.49 ± 0.07[b]

TV; Testicular volume, TS; Thickness of scrotal skin, WTS; Width of total scrotum
Values with different superscripts in the same column are significantly different ($P < 0.05$)

Table 4: The effect of year seasons on sexual behaviors of Arabian stallions

Seasons	No. of M/E	RT (min)	T to erection (min)	T to Ej (min)	Ej times (sec)
Spring	2.33 ± 0.14[c]	4.17 ± 0.2[c]	2.73 ± 0.11[c]	2.65 ± 0.16[c]	60.41 ± 1.79[c]
Summer	1.78 ± 0.13[d]	2.93 ± 0.14[d]	1.84 ± 0.1[d]	1.74 ± 0.09[d]	45.85 ± 1.87[d]
Autumn	2.85 ± 0.15[b]	6.09 ± 0.27[b]	3.2 ± 0.12[b]	3.87 ± 0.15[b]	85.93 ± 1.81[b]
Winter	3.7 ± 0.15[a]	8.48 ± 0.37[a]	3.7 ± 0.19[a]	5.69 ± 0.29[a]	96.85 ± 1.71[a]

No. of M/E; Number of mounts/ejaculation, RT; Reaction time, T; time, Ej; Ejaculation
Values with different superscripts in the same column are significantly different ($P < 0.05$)

RESULTS

Monthly evaluation of semen parameters, testicular biometry and sexual behavior of Arabian stallions

Data in Table 2 showed that the maximum ($P < 0.05$) TSV, GV, GFV and Con/ejac were recorded during June and July, while the minimal values were recorded during December, January and February. Moreover, the maximum ($P < 0.05$) Liv %, Motil % and Psm % were recorded during August, July and June, while the minimal values were recorded during December and January. Contrary, the Con/ml did not show significantly ($P > 0.05$) variation throughout the year.

Moreover, the maximum ($P < 0.05$) length, width, height and volume of testes were recorded during August, July and September, while the minimal values were recorded during December and January (Figure 1). Similarly, the maximum ($P < 0.05$) width of total scrotum was recorded during August and July, while the minimal values were recorded during December, February and January (Figure 1). Contrary, the thickness of scrotal skin was significantly lower during August, July and June ($P < 0.05$) compared with other months (Figure 1). Furthermore, the number of mounts per ejaculation, reaction time, time to erection, ejaculation times and time to ejaculation were significantly ($P < 0.05$) lower during August, July and June compared with December, February and January (Figure 2).

Effect of season on testicular biometry and sexual behavior of Arabian stallions

Data in Table 3 showed that the length, width, height and volume of testes were significantly ($P < 0.05$) lower in winter season compared with other seasons. Similarly, the width of total scrotum was significantly ($P < 0.05$) lower in winter season compared with other seasons. However, the thickness of scrotal skin was significantly ($P < 0.05$) higher in winter season compared with other seasons.

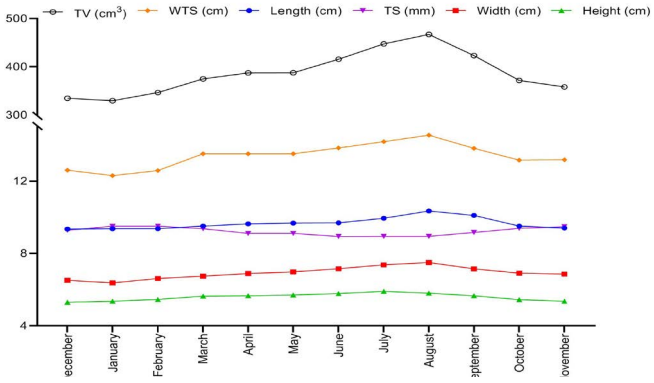

Figure 1: Monthly testicular biometry of Arabian stallions. TV; Testicular volume, WTS; Width of total scrotum, TS; Thickness of scrotal skin,

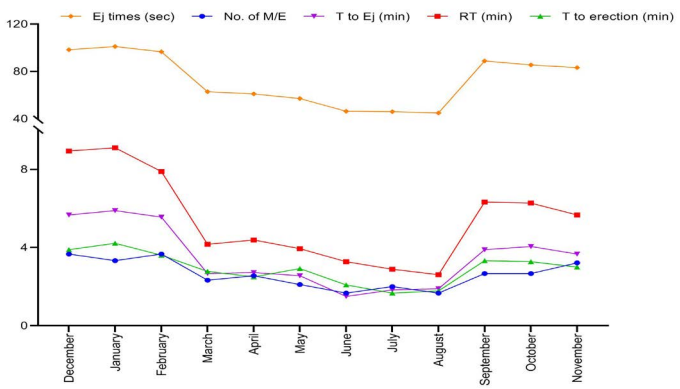

Figure 2: Monthly sexual behaviors of Arabian stallions. Ej; Ejaculation, No. of M/E; Number of mounts/ejaculation, T; time, RT; Reaction time.

Table 5: Pearson correlation coefficients between testicular biometry and semen parameters of Arabian stallions

	TSV	GV	GFV	Con/ej	Liv %	Motil %	Psm %
Length (cm)	0.447*	0.379*	0.426*	0.310*	0.383*	0.379*	0.406*
Width (cm)	0.479*	0.384*	0.461*	0.397*	0.481*	0.531*	0.510*
Height (cm)	0.619*	0.525*	0.590*	0.428*	0.442*	0.470*	0.478*
TV (cm^3)	0.558*	0.490*	0.528*	0.424*	0.503*	0.522*	0.517*
WTS (cm)	0.515*	0.376*	0.504*	0.425*	0.469*	0.527*	0.538*

TV; Testicular volume, WTS; Width of total scrotum, TSV; Total semen volume, GV; Gel volume, GFV; Gel-free semen volume, Con/ej; Concentration/ejaculate x 10^9, Liv; Livability, Motil; Motility, Psm; Progressive sperm motility

* Correlation is significant at the 0.01 level

Table 6: Pearson correlation coefficients between sexual behaviors and semen parameters of Arabian stallions

	TSV	GV	GFV	Con/ej	Liv %	Motil %	Psm %
No. of M/E	-0.493**	-0.302**	-0.494**	-0.355**	-0.255**	-0.313**	-0.351**
RT (min)	-0.512**	-0.340**	-0.507**	-0.426**	-0.406**	-0.488**	-0.530**
T to erection (min)	-0.491**	-0.371**	-0.478**	-0.355**	-0.225*	-0.342**	-0.424**
T to Ej (min)	-0.545**	-0.365**	-0.540**	-0.376**	-0.405**	-0.492**	-0.506**
Ej times (sec)	-0.580**	-0.408**	-0.570**	-0.451**	-0.468**	-0.538**	-0.588**

No. of M/E; Number of mounts/ejaculation, RT; Reaction time, T; time, Ej; Ejaculation, TSV; Total semen volume, GV; Gel volume, GFV; Gel-free semen volume, Con/ej; Concentration/ejaculate x 10^9, Liv; Livability, Motil; Motility, Psm; Progressive sperm motility

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

Additionally, data in Table 4 showed that the number of mounts per ejaculation, reaction time, time to erection, time to ejaculation and ejaculation times were significantly ($P < 0.05$) lower in Summer season compared with other seasons.

Correlation between testicular biometry and semen parameters of Arabian stallions

Interestingly, there was a significant correlation between the spermatic parameters and the testicular parameters (Table 5), especially between the testicular height and the total semen volume (r = 0.62). Similarly, there was a significant correlation between the spermatic motility and the testicular biometry.

Correlation between sexual behaviors and semen parameters of Arabian stallions

There was a significant correlation between the spermatic parameters and the sexual behaviors (Table 6), especially between the ejaculation times (sec) and the total semen volume (r = -0.58). Similarly, there was a significant negative correlation between the spermatic motility and the sexual behaviors.

DISCUSSION

Males have been recognized to show variation in their reproductive performance as size of testis, production of sperm and reproductive behaviour (Gerlach and Aurich, 2000). The seasonal change in length of day considered as the main regulating factor of reproductive activity (Chemineau et al., 2008). Stallions, a long-day breeders, have a maximum reproductive capacity during the seasons with prolonged photoperiod. Some studies suggest that reproductive activity of equine varies according to the local photoperiod length, as the fertile period in the Northern Hemisphere takes place from April to September (Hughes et al., 1975). In the current study we evaluated the influence of year months and seasons on testicular biometry, sexual behaviors and semen parameters of Arabian purebred stallions under subtropical conditions in Kuwait. Similar studies investigated influence of day length on seasonal reproductive activity of stallions (Clay et al., 1987).

Our results demonstrated that, the length, width, height and volume of testes as well as the width of total scrotum were higher during August and July. This was comparable with previous reports showed that testicular size, and sperm production were significantly affected by season, and the best values were recorded in May, June, or July (Pickett, 2018). We also reported that the testicle biometry as well as the width of total scrotum were higher during summer season compared with other seasons, which is in agreement with previous studies reported that stallions have higher testicular measurements during summer and spring, which subsequently leads to increase sperm production and libido (Hind et al., 2021).

Meanwhile, we found that the width of total scrotum and testicle biometry were significantly higher during breeding (spring and summer) season, which is matching with previous finding reported that testicular size, production of sperm and libido increase during breeding season (Clay et al., 1987). In the same way, testicular weight was reported to increase during the periods corresponding to the photoperiod variations (Jones and Berndtson, 1986; Najjar et al., 2012).

The seasonal changes in sperm production has been recorded (Clay et al., 1987). Our results showed that the semen quality was higher during June and July. These results are in line with previous studies reported that seminal parameters as semen volume, sperm concentration and sperm motility increase during the breeding season (Janett et al., 2003). Similarly, it has been reported that size of testis, sperm production and libido increase during the breeding season (Johnson, 1985; Clay et al., 1987). It has been reported that environmental temperature may also play a role in semen quality, as gel-free volume was reported to be highest in spring and summer and lowest in winter due to positive influences of environmental temperature on metabolism and many enjoyable horse-related activities are available during summer months (Janett et al., 2003; Schmidt et al., 2017; Shawki et al., 2021).

Assessment of the sexual behavior of stallion is critically important as it is closely linked to reproductive efficiency (Rua et al., 2015). The sexual beheviour is the highest in summer and spring, however it is usually reduced in winter (Waheed et al., 2015). Additionally, sexual behavior, ejaculate volume, total sperm output and motility were found to be higher during summer and spring compared to winter (Suliman et al., 2020). These findings are matching with our finding reported that the number of mounts per ejaculation, reaction time, time to erection, ejaculation times and time to ejaculation were significantly lower in summer season compared with other seasons. We also found a significant correlation between the spermatic parameters and the sexual behaviors, such effect is also reported by previous study found that number of mounts as well as collection time greatly affected fresh semen fertility (Najjar et al., 2010). Also, the results of current study demonstrated that there was a significant correlation between the spermatic parameters and the testicular parameters. Which is in line with previous studies reported that total scrotal width is positively correlated with daily sperm output (Thompson et al., 1979).

CONCLUSION

From the presented results, testicular biometry can be used for assessment of breeding soundness of Arabian Stallions under subtropical conditions. Data generated from our study provided valuable information on the stallions' reproductive efficiency in the subtropics and devise superior strategies for equine breeding selection especially for artificial insemination program in a subtropical environment. The testicular biometry and sexual behavior during spring and summer showed better characteristic than that during winter.

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CONFLICTS OF INTEREST

There is no conflicts of interest.

AUTHOR CONTRIBUTION

All the authors contributed to this manuscript; GS, TMB and AAA conceived and designed the experiments. AAA and AMS performed the experiments. AM and GS analyzed the data. AM wrote the first draft of the manuscript, revised and edited the manuscript.

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