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Theriogenology

Some Parameters of Stallion Semen Ejaculates During Different Seasons

Mahmoud A. Sultan*, Emad M. Abd El-Razek, Ahmed H. Zaghloul, Tamer M. Genedy, Hamed T. Elbaz

Department of Theriogenology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Menofia 32897, Egypt

**Corresponding author:* sultanmahmoud2019@gmail.com *Received: 2/1/2022 Accepted: 12/1/2022*

ABSTRACT

The aim of the present study was to characterize the effect of season on some parameters of native Egyptian stallion semen ejaculates. Semen was collected from six mature healthy stallions once per season. The parameters of evaluation include macroscopical (volume, color and consistency) and microscopical examination (motility, live %, SCC, sperm morphology and abnormalities) of stallion semen characters. Results revealed that the gel free volume, total sperm per ejaculate, normal sperm morphology and longevity of stallion semen at room temperature 20-25°C in non-breeding season and 32-38°C in breeding season and at 38°C were significantly higher ($P<0.01$) in breeding season than in non-breeding season while abnormal sperm percentage was significantly higher ($P<0.01$) in non-breeding season than in breeding season. Furthermore, the gel portion of the ejaculate, live sperm percentage, motility of sperm and sperm cell concentration were not showed any significant difference between breeding season and non-breeding season. Conclusion, the season has a direct significant effect on the volume of ejaculate, total sperm per ejaculate, normal sperm percentage and abnormal sperm percentage.

Keywords: Season, Semen, Sperm and Stallion.

INTRODUCTION

Artificial breeding in equine doesn't have a great development as in other species, which may be arise to avoid the problems of inbreeding and to preserve the bloodlines and pedigree (Alvarez et al., 2014). Equine directs their reproductive activity into a season, which is advantageous for their young. Seasonal changes in the day length are the main cause of the decrease and increase in the horses sexual activity (Janett et al., 2003; Gamboa et al., 2010). Horses are long day seasonal breeder animals, with maximum sexual activity in summer and spring (Chemineau, 2008). Deichsel et al. (2016) reported that characteristics of semen were improved in stallions when there is a 8 h darkness and a 16 h light in northern hemisphere winter, thereby providing a physiological explanation for changes in characteristics of semen, likely mediated through effects of increased light on secretion of GnRH (Aurich, 2016). In addition, there are evidences that sperm production of stallions decreases in the non-breeding season (Brito, 2007). While, Wrench et al. (2010) reported that, there is no differences between the breeding and the non-breeding season concerning to the volume of the ejaculate,

the concentration of the ejaculate, the total number of movable spermatozoa or the progressive motility of the ejaculated sperm by stallions. However, different studies in some breeds of stallions showed that dimensions of testicles and season don't affect the semen quality parameters but affect volume of the ejaculate (Vidament et al., 2006). Variations in the characters of stallion semen have been found to be affected by frequency of ejaculate collection and season of the year, so that the increase in frequency of ejaculation associated with a decrease in the concentration of sperm and total number of sperm (Janett, et al., 2003). Maximum sexual performance in stallions can be achieved through maintenance of their normal reproductive behavior and libido through obtaining high quality of ejaculate by adjusting the frequency of semen collection (Sieme et al., 2004; Aurich, 2016). Therefore, the aim of this study was to characterize the effect of season (breeding and non-breeding) on some parameters (volume, color, consistency, motility, live %, SCC, sperm morphology and abnormalities) of native Egyptian stallions semen.

MATERIALS AND METHODS

This study was assessed and agreed by the Animal Care and Welfare Committee Ethics, University of Sadat City, Egypt.

Animals

The present study was done on a total number of six mature healthy Egyptian native breed stallions (8-12 years old). The study was carried out at Shamma village, Ashmoun, Menofia, Egypt during different seasons (November 2020 to August 2021). Animals were raised and housed together in a pen fed on (corn and bran), water provided adilibtum. No fertility problems were detected after complete comprehensive reproductive soundness examination before experiments. The reproductive history revealed that, stallions were used in natural mating. Animals were kept according to experimental design and semen was collected once per season.

Semen collection

Semen was collected from these stallions using a pressurized, pre-warmed (42-45℃) and lubricated home-made artificial vagina and a secured mare not in estrus was used as a dummy in Autumn and Winter seasons (non-breeding season) as well as a fully estrus female used as a dummy in Spring and Summer seasons (breeding season) (Hanulakova et al., 2012). Two ejaculate were collected from each stallion (the first ejaculate was collected from stallions after several false mounting). A sterile gauze was used as a filter to remove gel fraction of the ejaculate.

Semen evaluation

Immediately after the semen collection, the semen was filtered through the gauze then the semen was examined for the basic semen characteristics which include:

Macroscopic evaluation

The volume of the ejaculate (gel-free portion) and the gel portion were measured using graduated plastic tubes (50 ml) (Sieme et al., 2004). The color of the ejaculate was examined through visual examination (Sieme et al., 2004). The consistency of the ejaculate was examined through visual examination (Samper, 2009).

Microscopic evaluation

Sperm individual motility was assessed by Turner (2005). Assessment of the viability of the sperm using eosin-nigrosine stain as recorded by Evans and Maxwell (1987), assessment of sperm cell concentration ($x10^6$/ml) through direct cell count using neubauer-haemocytometer (Bailey et al., 2007). Calculation of total sperm per ejaculate ($x10^9$) by multiplying the sperm cell concentration ($x10^6$/ml) in the ejaculate volume (Samper, 2009). Assessment of sperm morphology and abnormalities using alkaline crystal violet stain as described by

(Dott and Foster, 1972). Assessment of sperm longevity at room temperature and at 38°C according to Bradecamp (2011).

Statistical analysis

Data are represented as mean ± standard deviation. We checked normal distribution using Shapiro-Wilk test and checked homoscedasticity using Levene's test. Then, significance was tested using t-test to compare breeding and non-breeding season semen parameters. All statistical analysis and graphs were performed using RStudiov1.3.1093 (RStudio Team, 2020) and R programming language v4.0.3 (R Core Team, 2020). Differences between groups were considered significant when (*p*-value < 0.05).

RESULTS

Macroscopic examination of stallion fresh semen

Macroscopically characteristics of native stallions semen as presented in (Table). The gel free volume was significantly higher (P<0.01) in the breeding season than in the non-breeding season while the gel volume was not significant as presented in (Fig.1 and Fig.2). The color of the examined stallions semen was whitish gray and the consistency of semen was watery with pH around 7.2.

Microscopic examination of stallion fresh semen

Microscopically characteristics of native stallions semen as shown in table. The normal sperm percentage and the total sperm per ejaculate were significantly higher (P<0.01) in the breeding season than in the non-breeding season as presented in (Fig.3 and Fig.4). While, there is no significant differences in (sperm cell concentration, viability and motility) of sperm during breeding and non-breeding season. However, the abnormal sperm percentage was significantly higher (P<0.01) in non-breeding season than breeding season as shown in (Fig. 5).

Table (1): Parameters of fresh semen of stallions during breeding and non-breeding season:

Parameter	breeding season (Mean ± SEM)	Non-breeding season (Mean ± SEM)
Volume (ml)	102.4 ± 15.8	63.8 ± 10.4 ***
Gel portion (ml)	8.3 ± 9.5	3.7 ± 4.3
Live sperm (%)	72 ± 5.3	66 ± 7.04
Motility (%)	75 ± 5	68.3 ± 8.16
SCC ($x10^6$/ml)	146 ± 22.2	128.8 ± 29.2
Total sperm/ejaculate	15.1 ± 4.1	6.94 ± 2.4 **
Normal sperms (%)	73.3± 3.6	57.2 ± 3 ***
Abnormal sperms (%)	26.7± 3.6	42.8 ± 3 ***
Longevity at RT (min)	13 ± 4.1	5 ± 0 *
Longevity at 38ºC (min)	18 ± 1.3	14.5 ± 3.5 *

Data are presented as (mean ± SEM).

*mean significance (P<0.05),

**mean significance (P<0.01),

***mean significant difference (P<0.001).

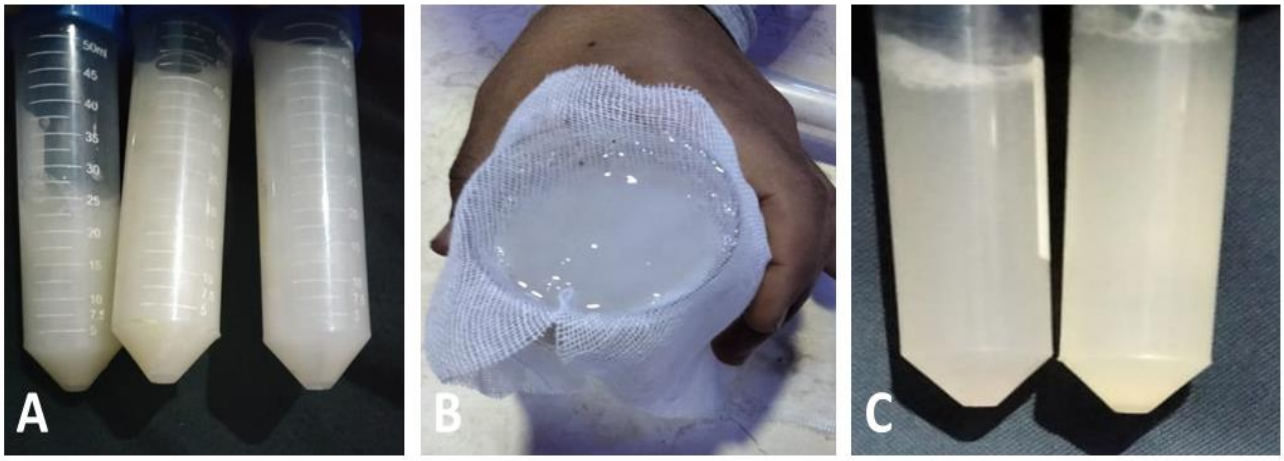

Fig.1. Stallion semen ejaculates after collection by AV. (A) Semen ejaculates with gel (B) Semen ejaculate showing gel trapped on a piece of gauze(C) Semen ejaculates without gel.

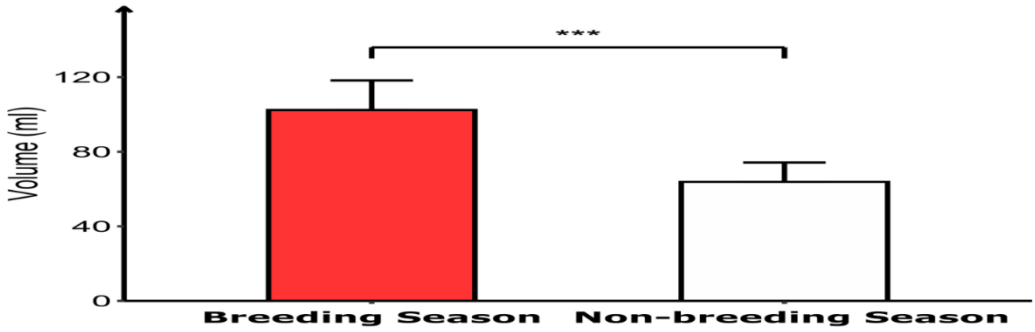

Fig. 2. The effect of season (breeding and non-breeding) on volume of stallion's semen.

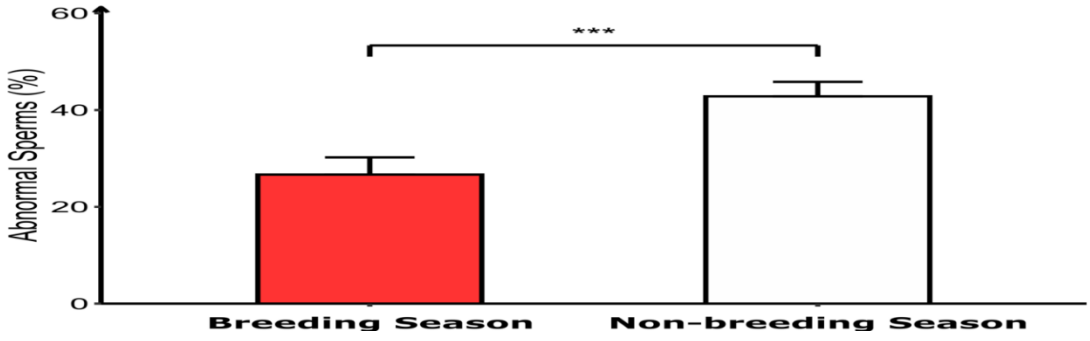

Fig. 3. The effect of season (breeding and non-breeding) on total stallion's sperm per ejaculate.

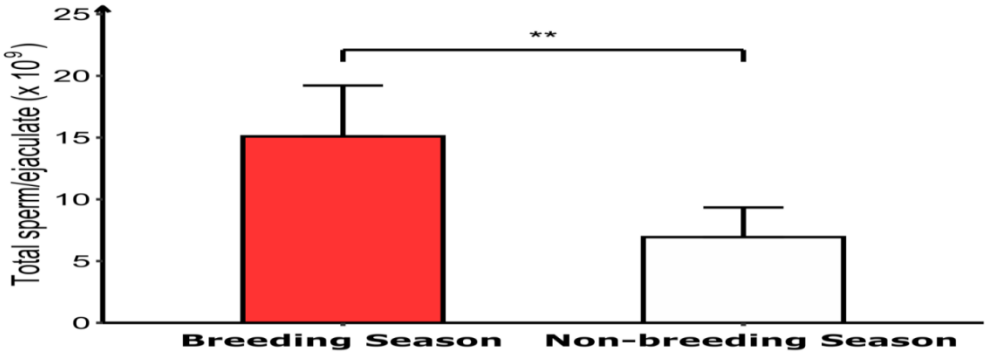

Fig. 4. The effect of season (breeding and non-breeding) on normal stallion's sperm percentage.

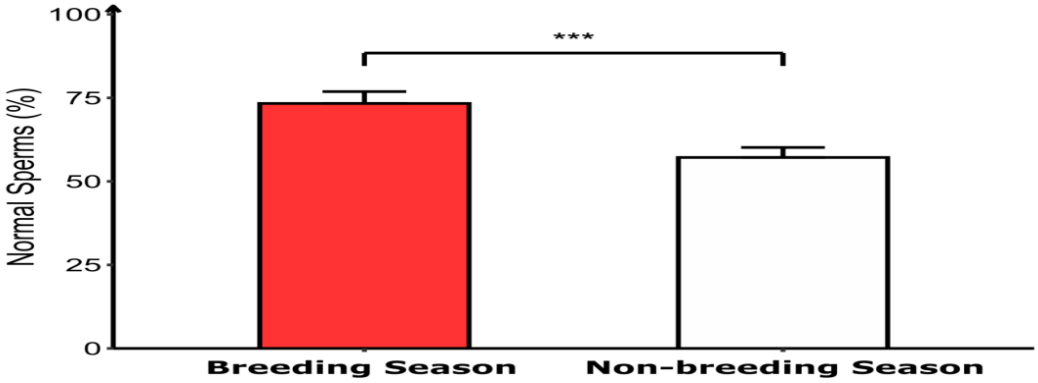

Fig. 5. The effect of seasononabnormal stallion's sperm percentage.

DISCUSSION

In the present study, it was found that the average volume of semen from stallions collected during the breeding season was significantly higher than the average volume of semen from stallions collected during the non-breeding season. This result is in a harmony with the results obtained by Love et al. (2000); Blanchard et al.(2001); Janett et al. (2003); Robalo Silva et al. (2007) and Cazales et al. (2008). While, Suliman et al. (2020) reported that no seasonal variation between stallions in the volume of the ejaculate in the breeding season and the non-breeding season. The average volume of semen of stallions was in a range of 50-130 ml and this was in accordance to result obtained by (Samper, 2009) who reported that the average semen volume was 60-120 ml. This difference between seasons may be attributed to the age of stallions, size of the testes, reserve capacity of the sperm in the epididymis, nutrition, frequency of collection, libido, technique of collection and season of the year.

The gel volume of the ejaculate varies from no gel to 20 ml in different seasons. This result is in agreement with Morel (2008) who reported that the volume of the gel enormously varies from no gel at all to 80 ml. The ejaculate gel volume was not significantly different in the breeding season and the non-breeding season. This result is in agreement with Suliman et al. (2020). While, Morel (2008) reported that the ejaculate gel volume was higher in the breeding season than non-breeding season. I think this difference may be attributed to libido, stallion age and the breeding season.

Macroscopic evaluation of the color of stallion semen reveals that the color of the ejaculated stallion semen in different

seasons is white or whitish-gray. This result is in agreement with results obtained by Sieme et al. (2004) and Feradis (2010). Macroscopic evaluation of the consistency of stallion semen revealed that the consistency of stallion semen is watery. This data is in agreement with Samper (2009) who reported that the semen of stallions is very diluted and watery.

Only semen with live sperm percentage more than 60% was accepted. This was in accordance with (Morel, 2008) who reported that the acceptable percentage of live sperm must be more than 60%. There is no significant difference between stallions in the live sperm percentage in the breeding season and non-breeding season. This result is in agreement with the results obtained by (Vidament et al., 2006).

In relation to the motility of the semen of stallions, it is found that the motility during the breeding season was stable. When comparing the motility during breeding season and non-breeding season, it is found that there is no significant difference observed in the motility in breeding season and non-breeding season. These data is in a harmony with the results reported by (Vidament et al., 2006; Robalo Silva et al., 2007 and Wrench et al., 2010). These data is not in agreement with Janett et al. (2003) who reported that the motility of stallion sperm was higher in breeding season than in non-breeding season. While, Warnke et al. (2001) reported that the motility of stallion sperm is higher in winter (non- breeding season) than in summer and spring (breeding season)

Evaluation of sperm cell concentration showed that no significant difference between stallions in breeding season and non-breeding season. These data was in a harmony with the results reported by Suliman et al. (2020). These data is not in agreement with results obtained by (Janett et al., 2003 and Wach-Gygax et al., 2017) who reported that the sperm cell concentration of stallion ejaculate was lower in the summer (breeding season) than in winter (non-breeding season). The sperm cell concentration is in a range of 100 million to 170 million per ml. this result is in agreement with Samper (2009) who reported in his study that the sperm cell concentration was 100-350 x 10^6 which fell in range of 100-150 x 10^6/ml if collection of semen was performed regularly.

It is found that total sperm per ejaculate was higher in the breeding season than in the non-breeding season. This result was in agreement with Janett et al. (2003). However, Suliman et al. (2020) reported that no significant difference was present in the volume of the ejaculate and the sperm cell concentration between different seasons so no difference in the total number of sperm of stallion ejaculates in the non-breeding season and the breeding season. These differences in the concentration of sperm cells may be related to season of the year, medication, nutrition and frequency of collection.

It is found that the percentage of normal stallion sperm was significantly higher in breeding season than in the non-breeding season. This result was in agreement with results in the study of (Suliman et al., 2020) that reported that morphological examination of stallion sperm in fresh semen of stallions showed that normal stallion sperm percentage was higher significantly in the breeding season than in the non-breeding season in fertile stallions. In contrast to that results, (Blottner et al., 2001 and Janett et al., 2003) reported that the percentage of normal stallion sperm was significantly higher in the non-breeding season than in the breeding season. The discrepancy may be due to effect of season, method of collection, frequency of collection and the skill of examiners.

The percentage of abnormal stallion spermis significantly higher in non-breeding season than in breeding season. This result was in a harmony with (Suliman et al., 2020)who reported the morphological examination of stallion sperm in fresh semen of stallions showed that abnormal stallion sperm percentages higher significantly in non-breeding season than in breeding season in fertile stallions. While, Janett et al., 2003 reported that the normal morphology of stallion sperm is lower significantly in non-breeding season than in breeding season and the major defects in stallion sperm morphology showed higher values in the breeding season than in the non-breeding season. In contrast results obtained by Kavak et al. (2004) who detected that there is no any relationship between the normal morphology of the stallion sperm and the fertility of stallions. Furthermore, presence of any abnormalities in stallion sperm belongs to the normal fertility of stallions.

It was found that the sperm remain viable and motile for a period range from 5 to 20 minutes either at room temperature or at 38°C. This result was in agreement with Blanchard (2007) who reported that the longevity and the livability of raw un-extended semen of stallion at room temperature was very short which reached to few minutes only. The longevity of stallion extended semen at room temperature was not more than six hours. This result is in agreement with (Bradecamp, 2011) who reported that stallion extended semen had the ability to become viable and had the ability to have motility at least 10% after 6 hours of storage at room temperature.

Conclusion

The season has a significant effect on the volume of ejaculate, total sperm per ejaculate, normal sperm % and abnormal sperm % andthe present study provides basic parameters for semen evaluation in native Egyptian stallion during different seasons:

Conflict of interst

The authors declared that no conflict of interests exists.

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