EFFECTS OF SEASONAL VARIATIONS ON PHYSICAL, MOLECULAR AND FREEZABILITY OF FRIESIAN AND BUFFALO BULL SEMEN

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ABSTRACT

Seven mature Friesian and seven mature buffalo bulls, proven to be free of venereal and infectious diseases, were subjected to twice per week semen collection by an artificial vagina throughout four consecutive seasons (Year 2002-2003). Ejaculates were initially assessed for physical characteristics and high quality specimens were pooled within a species, processed and frozen. Individual sperm motility percentages were recorded after dilution, equilibration and thawing. Additionally an aliquot of seminal plasma representing species within a season was subjected to sodium dodecyl sulphate electrophoresis (SDS – PAGE) to monitor the changes in type and intensity of seminal plasma peptides.

Mean ejaculate volume was higher (P<0.05) in Friesian (5.1ml) than in buffalo (3.5ml) bulls. However, season has no significant effect on ejaculate volume. Sperm cell concentration and percent progressive motility were higher (P<0.05) for both buffalo and Friesian bulls during winter and autumn than during summer. Percent of post thaw survival was lowest during summer (48.3%) as compared with that obtained in winter (66.7%), spring (65.4%) or autumn (61.7%). Percent of post thaw sperm survival was higher (P<0.05) for Friesian (64.6%) than buffalo (56.6%) bulls. Number of peptide bands in seminal plasma of Friesian bulls were higher (16, 15, 15 and 17) than in buffalo (10, 11, 12 and 12) for seminal plasma of winter, spring, autumn and summer, respectively. There appears to exist one or two more peptide bands in summer than in winter seminal plasma. A darker band with molecular weight of 17.9 kDa was found in Friesian, but not in buffalo seminal plasma. Moreover, there are two more peptide bands (Mwt, 23.4 and 32 kDa) found in Friesian seminal plasma which were absent in buffalo. On the contrary, there existed a band of 14 kDa in buffalo, but not in Friesian seminal plasma.

This study concluded that climatic season has a significant influence on the physical and chemical characteristics of semen and, in turn, affecting its freezability and subsequent fertility.

***Key words:** Season, Friesian, buffalo, sperm, freezing, peptide pattern.*

INTRODUCTION

Freezing of semen collected from buffalo has been a focus of several A.I. centers in Egypt. Frozen semen of elite bulls can be used as a tool for improving the productive and reproductive performance of herds.

Fertilizing capacity of semen ejaculate depends not only on sperm characteristics, but also on the seminal plasma components surrounding sperm cells. Till now, there is no single simple laboratory test or practical and accurate method to quantitatively assess an ejaculate to determine its level of fertility. Available microscopic and biochemical tests for semen quality are primarily indicators of sperm viability but not of freezability and fertilizing competence. In dairy bulls, there are proteins that are prevalent in semen from bulls of above average fertility and different proteins that are abundant in seminal fluids from bulls of below average fertility. Seasonal variations have been shown to influence the sperm characteristics and seminal plasma composition (Garner and Hafez, 2000; Eweda, 2001; Dowidar, 2002 and El-Sherbieny, 2004). Also, the variability of the forages and feedstuffs could have an impact on the semen quality (Dhami *et al.*, 1987 and Eweda, 2001). Therefore, the present study aimed at : (a) investigating the effect of seasonal changes on semen characteristics and freezability of Friesian and buffalo bulls and (b) correlating the morphological and physical sperm characteristics with the changes in the seminal plasma peptide composition and behaviour.

MATERIALS AND METHODS

Location

The present study was conducted between September 2002 and August 2003 at the International Livestock Management Training Center (ILMTC), Sakha, Animal Production Research Institute (APRI), Agricultural Research Center, Kafer El-Sheikh, Egypt.

Semen collection and evaluation

Semen ejaculates were collected twice a week throughout the course of the experiment (one year) by a sterile artificial vagina from seven mature Friesian and seven mature Egyptian buffalo bulls. Animals were free from venereal diseases and clinically normal. Bulls were housed in open yards and fed and treated according to the routine managerial system of the experimental farm. Semen ejaculates of each species were individually evaluated for volume, progressive motility, sperm concentration, percent live sperm and percent total abnormalities. Seminal plasma were separated by centrifugation of semen at 3000×g for 30 minutes. Plasma samples were harvested and stored in clean labeled Eppendorff tubes at –20°C till used for analysis.

Semen freezing technique

Accepted ejaculates (advanced motility ≥70%) for each species were pooled and processed for freezing technique which was previously described by Abd-El-Azeez (1988). Individual sperm motility percentages were recorded after dilution, equilibration and thawing according to Aziz *et al.* (1994). The thawing procedure was performed in a water bath at 38°C for 30 sec.

Seminal plasma peptide pattern

Vertical polyacrylamide gel slab electrophoresis in the presence of sodium dodecyl

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sulfate (SDS-PAGE) at pH 8.6 was carried out with Mini-Protean II cell (Bio-Rad, CA, USA) at 120 volt and room temperature for 2 hours. The gel used (0.75mm thick) consisted of 4.5% T stacking gel and 10%T separating gel (T% is an expression representing the concentration of acrylamide plus bisacrylamide in the gel). The electrode and migration buffers consisted of 0.19M glycine and 0.024M Tris and 1% SDS at pH 8.6. After electrophoresis, proteins were localized in gels using 0.1% coomassie blue (Laemmli, 1970).

Peptide molecular weight determinations

Molecular weights (kDa) of separated proteins were estimated according to the method of Weber and Obstorn (1969) after separation on SDS-PAGE using standard protein marker.

Statistical analysis

Data of physical semen characteristics were analyzed by the Least Square Analysis of Variance (GLM, ANOVA) using SAS statistical package (SAS, 1995). A Least Significant Differences (LSD, Steel and Torrie, 1980) was used to compare between Friesian and buffalo means. Results were considered significant at $P< 0.05$ or less.

RESULTS AND DISCUSSION

Results of fresh semen characteristics are shown in histograms (Fig. 1). Semen ejaculate volume (Fig. 1a) was not different between seasons within a bull species. However, mean ejaculate volume was significantly ($P<0.05$) higher in Friesian (5.1 ml) than buffalo (3.5 ml) bulls. Similar recent findings were reported by Dandoush (2002), Ibrahim (2003) and El-Sherbieny (2004). Such trend could be due to the variations in the genetic makeup between species (i.e. different functional capacities of the accessory sex glands between Friesian and buffalo bulls).

Sperm cell concentration was significantly ($P<0.05$) higher for both species during winter and autumn months, but summer semen ejaculates exhibited the lowest sperm concentrations for both species (Fig. 1b). The values of sperm concentration in summer amounted at 66% of their respective values in winter for both species. Effects of heat stress can be acute or chronic. Heat stress has been found to have an adverse effect on spermatogenesis and sexual behaviour (Garner and Hafez, 2000). Also, Ax *et al.* (1987) found decreases in concentrations of sperm, percent motility and live sperm with corresponding increases in the frequency of abnormal sperm in summer months. Moreover, sperm concentration was significantly ($P<0.05$) higher in buffalo than in Friesian semen. This indicates a negative relationship between the ejaculate volume and sperm concentration within a species as reported by Garner and Hafez (2000) and El-Sherbieny (2004).

Percentage of progressive motility was highest during winter (79.3%) and lowest during summer (71.0%) months (Fig. 1c) for both species. These values were similar to those reported by Bhosrekar *et al.* (1992). The value of progressive motility was significantly ($P<0.05$) higher in Friesian (77.9%) than in buffalo (73.4%) semen. These results are in agreement with those reported by El-Keraby *et al.* (1995) and Ibrahim (2003).

The overall mean values of live sperm and total abnormalities were significantly ($P<0.05$) affected by the season of the year. Values of percentage of live sperm were 80.5, 86.5, 82.0 and 75.2% during autumn, winter, spring and summer seasons, respectively. On the other hand, percentage of abnormal sperm was highest during summer (15.6%) and lowest during winter season (6.9%). These results are in agreement with those reported by Ax *et al.* (1987) on cow's bulls and Dowidar (2002) on buffalo's bulls. Silva and Cosagrande (1976) reported that the differences in semen characteristics may be due to the effects of heat stress during hot season on testicular function. However, no significant differences were found between the two species in the percentage of live and abnormal sperm but there was a tendency of higher percentages of live and abnormal sperm in Friesian (81.9 and 11.2%) than buffalo (80.2 and 10.1%) semen, respectively. Similar trend in buffalo and Friesian was observed by Ibrahim (2003). Total number of sperm per ejaculate and total number of motile sperm/ejaculate were highest during winter and lowest during summer months for both species (Fig. 1d&e).

Freezing of semen caused substantial ($P<0.05$) reduction in sperm motility for both species during different seasons (Fig. 2). The highest sperm survival was found during winter (66.7%) and spring (65.4%) and the lowest was found during summer (48.3%) months. These results agree with the results of Aziz *et al.* (1994) and Dowidar (2002) on buffalo bulls. Moreover, sperm motility and survival percentages of processed semen were found to be higher ($P<0.05$) in Friesian than buffalo bulls (Fig. 2). The obtained results indicate the suitability of winter and spring months for freezing Friesian and buffalo semen, followed by autumn season. On the other hand, summer season was the lowest in maintaining post-thaw sperm motility in both species. Also, it has been found in ram semen that ejaculates collected during hot summer months had lower post-thaw motility as compared with those collected during cold winter (El-Bahrawy *et al.*, 2004). Differences between seasons may be due to the changes in seminal plasma compounds (Eweda, 2001 and Dowidar, 2002) and/or to the change in structure of the sperm cell membrane (Jones, 1973). Generally, the decrease of motility post freezing has been confirmed and attributed mostly to the destabilization of sperm membrane integrity (DeLeeuw *et al.*, 1993).

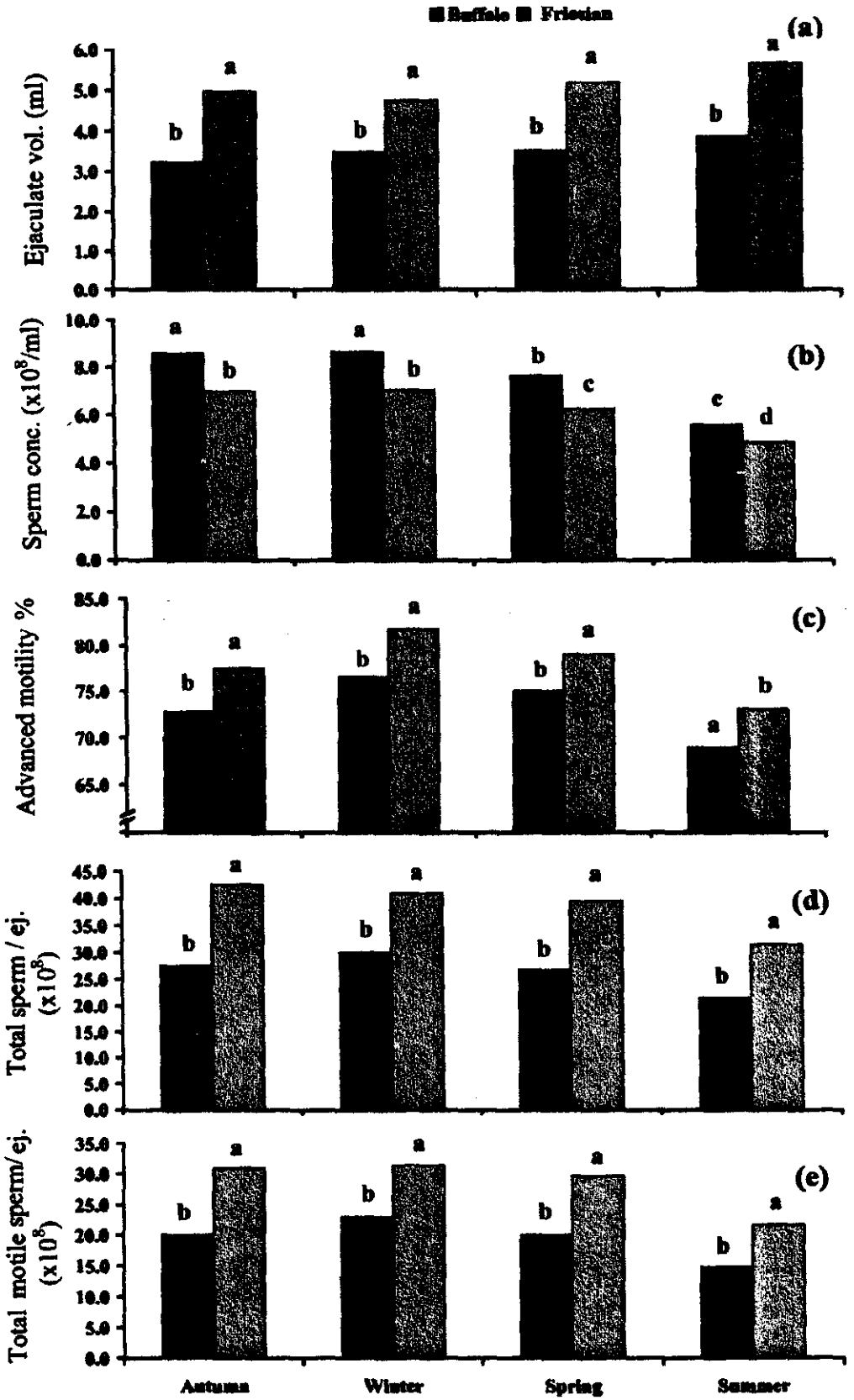

Fig. (1) : Effect of climate season on fresh semen characteristics of Friesian and buffalo bulls. Means with different letters differ significantly (P<0.05)

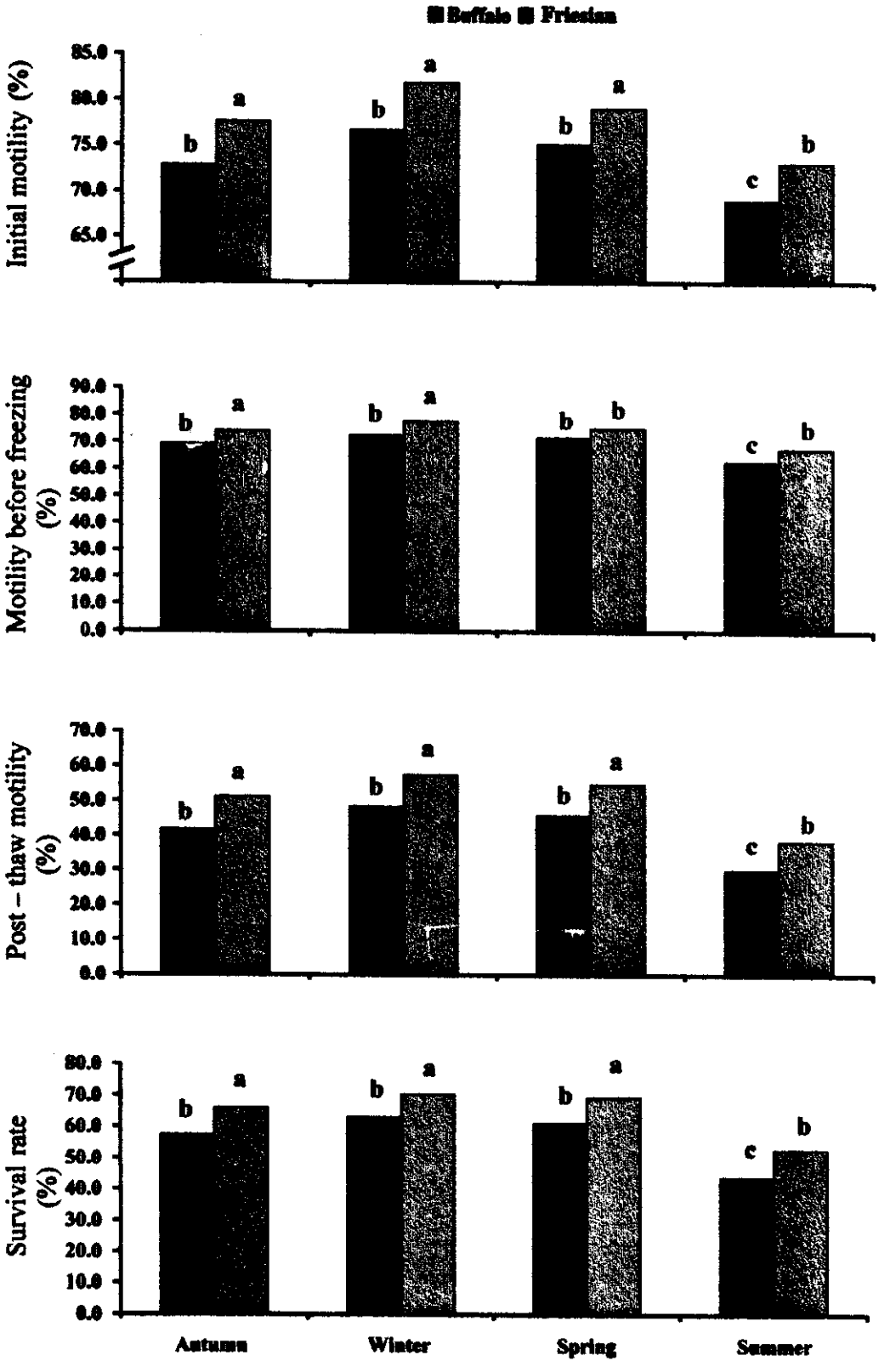

Fig. (2): Effect of bull specie and season on motility and survival of processed semen. Means with different letters differ significantly ($P<0.05$)

As shown in Fig. (3) the number of peptide bands in seminal plasma were higher in Friesian than buffalo bulls. Friesian seminal plasma exhibited about 5 more peptide bands than those existed in buffalo seminal plasma. There seems to exist a species and seasonal differences on the number and intensity of the peptide bands in seminal plasma. In Friesian seminal plasma there exist 16, 15, 15 and 17 peptide fractions in winter, spring, autumn and summer seasons, respectively. However, in buffalo seminal plasma there exist 10, 11, 12 and 12 peptide fractions in winter, spring, autumn and summer seasons, respectively. The most abundant protein which exist in Friesian seminal plasma and disappeared in buffalo seminal plasma was the 17.9 kDa molecular weight. Moreover, in Friesian seminal plasma there exist 2 bands (32 and 23.4 kDa) which were absent in the buffalo seminal plasma. On the other hand, a band of less than 14 kDa molecular weight was found in buffalo and disappeared in Friesian seminal plasma.

Generally, buffalo seminal plasma always has less number of peptide bands than those in Friesian seminal plasma. However, the excess two bands were found in summer seminal plasma of both Friesian and buffaloes. These two peptides are close in their molecular weights to acrosin inhibitors or trypsin, which play a vital role in the survival and fertilizability of spermatozoan. Free acrosin and trypsin presumably act by hydrolyzing the arginyl and lysyl bands in the zona pellucida, thereby altering the conformation of the receptor and depriving its ability to bind the spermatozoa (Mann and Lutwak-Mann, 1981).

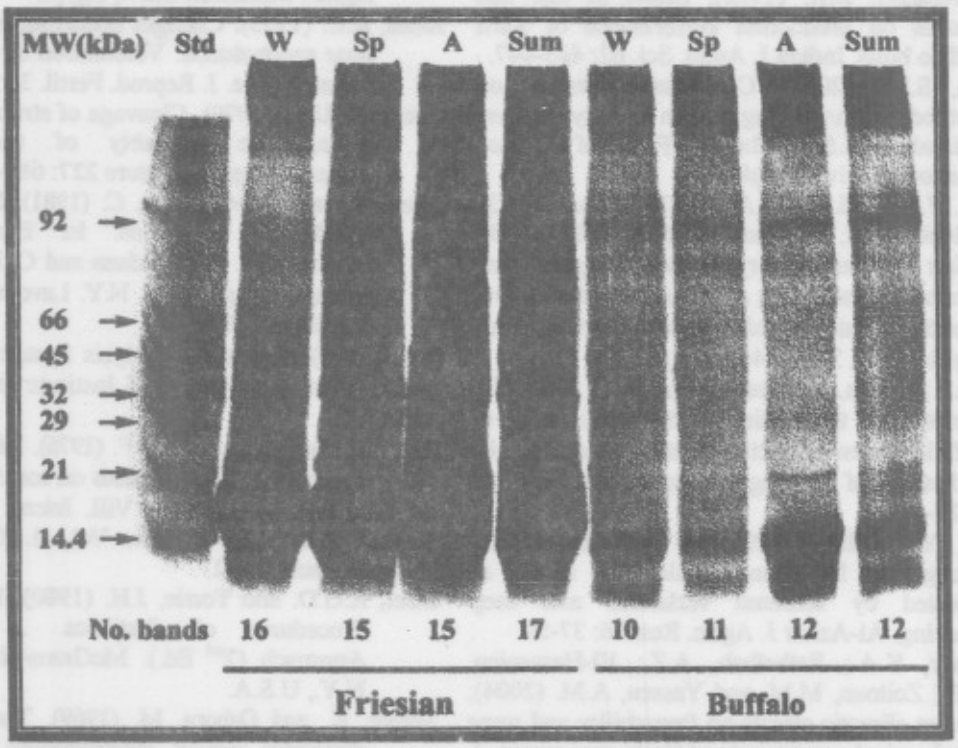

Fig. (3) : SDS polyacrylamide gel electrophoresis (SDS-PAGE, 10%T) of Friesian and buffalo bull seminal plasma throughout the four seasons of the year.
(Anode towards the bottom of the photo)
Lane 1: Standard marker
Lane 2-5: Friesian seminal plasma (winter, spring, autumn and summer seasons, respectively).
Lane 6-9: Buffalo seminal plasma (winter, spring, autumn and summer seasons, respectively).

In conclusion, this study indicated a species (genetic) and season (environment, especially feedstuffs) differences on the physical and biochemical characteristics of semen ejaculates. Winter and spring exhibited the best seasons to obtain high semen quality of Friesian and buffalo bulls which gave the best results post freezing. In both Friesian and buffalo semen there were one or two more peptide bands in summer than other season samples. These extra bands were of molecular weights closer to the acrosin inhibitor or trypsin enzyme, the proteins which impede the fertilizing ability of the sperm to the oocyte.

Therefore, this study suggests further research to investigate types and concentrations of the molecules present in seminal plasma and correlate such components with semen quality, freezability and fertilizing capacity of the semen ejaculate.

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الملخص العربى

تأثير التغيرات الموسمية على الصفات الطبيعية والجزيئية والقابلية للتجميد للسائل المنوي لطلائق الفريزيان والجاموس

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تمت هذه الدراسة بالمركز الدولي للتدريب على رعاية الحيوان بسخا، محافظة كفر الشيخ واستخدم فيها سبع طلائق أبقار فريزيان وسبع فحول جاموس كلها ناضجة جنسياً. تم جمع السائل المنوي منها بالمهبل الصناعي مرتين أسبوعياً لمدة عام بهدف دراسة التغيرات الطبيعية للسائل المنوي وكذلك أنماط البروتين في البلازما المنوية وقابلية الحيوانات المنوية للتجميد خلال مواسم السنة المختلفة لطلائق الفريزيان والجاموس.

ويمكن تلخيص النتائج فيما يلي :-

١- كان للموسم تأثيراً معنوياً (عند مستوى ٥%) على جميع الصفات الطبيعية المدروسة عدا حجم القذفة المنوية. أظهر موسم الشتاء أعلى جودة للسائل المنوي (أعلى قيم للحركة التقدمية والحي والتركيز وأقل قيم للشواذ الكلية) يليه موسم الربيع والخريف والصيف على التوالي.

٢- لوحظ تفوقاً معنوياً (عند مستوى ٥%) للسائل المنوي لطلائق الفريزيان عنه في طلائق الجاموس في حجم القذفة أو الحركة التقدمية والعكس بالنسبة لتركيز الحيوانات المنوية. بينما لم تكن الفروق معنوية في النسبة المئوية لكل من الحي والشواذ الكلية.

٣- كان للموسم تأثيراً معنوياً (عند مستوى ٥%) على قابلية الحيوانات المنوية للتجميد فقد انخفضت الحركة التقدمية بشدة بعد التجميد والإسالة خاصة في موسم الصيف وكانت الحيوية بعد الإسالة مباشرة ٦٦,٧، ٦٥,٤، ٦١,٧، ٤٨,٣% في موسم الشتاء والربيع والخريف والصيف على التوالي.

٤- وجد تفوقاً معنوياً (عند مستوى ٥%) للحيوانات المنوية لطلائق الفريزيان مقارنة بالجاموس في النسبة المئوية للحركة التقدمية بعد فترة الاتزان وكذلك بعد التجميد والإسالة وأن المتوسط العام للحيوية بعد الإسالة كان ٦٤,٦ مقابل ٥٦,٦% للأبقار والجاموس على التوالي.

٥- لوحظ اختلافات راجعة للموسم المناخي وأخرى مرجعها لنوع الطلوقة على عدد وكثافة الحزم البيتيدية الموجودة في البلازما المنوية، حيث كانت أعدادها ١٦، ١٥، ١٥، ١٧ حزمة بيتيدية في الأبقار مقابل ١٠، ١١، ١٢، ١٢ حزمة في الجاموس في عينات السائل المنوي لموسم الشتاء والربيع والخريف والصيف على التوالي.

ويظهر من هذه النتائج أن موسم الصيف في كلا النوعين (الأبقار، الجاموس) يحتوي على حزمتين بيتيديتين أكثر من المواسم الأخرى وهاتين الحزمتين يعتقد أنهما من العوامل المثبطة لنشاط الحيوان المنوي حيث أنهما تقتربان في وزنهما الجزيئي لكلا من مثبط الأكروسين والتربسين اللذان يلعبان دوراً حيوياً في تهيئة الحيوان المنوي للإخصاب.

٦- لوحظ وجود بروتين غزير في البلازما المنوية للأبقار وزنه الجزيئي يعادل ١٧,٩ كيلو دالتون وهذا غير موجود في عينات الجاموس. كما توجد حزمتين بيتيديتين وزنهما الجزيئي يعادل ٢٣,٤، ٣٢,٠ كيلو دالتون في البلازما المنوية للأبقار وهما غير موجودتان في البلازما المنوية للجاموس. وعلى العكس توجد حزمة بيتيدية وزنها الجزيئي أقل من ١٤,٠ كيلو دالتون في البلازما المنوية للجاموس وغائبة في عينات السائل المنوي لطلائق الفريزيان.

نستخلص من هذه الدراسة أن موسم السنة يؤثر على الصفات الطبيعية للسائل المنوي وأيضاً على التركيب الكيماوي لبلازما السائل المنوي والتي تلعب دوراً أساسياً في حيوية الحيوانات المنوية وقدرتها على الحفظ بالتجميد سواء في الأبقار أو الجاموس.

وتفتح هذه الدراسة آفاقاً جديدة لإجراء بحوث في مجال الكيمياء الحيوية وكيمياء المناعة لبلازما السائل المنوي للأنواع المختلفة من الحيوانات المزرعية وتحت الظروف البيئية المختلفة.