Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 13 Issue:2, (2020) ISSN: P-1999:6527 E-2707:0603

Effect of Monthly Variations on the Plasma Membrane, Acrosome and DNA Integrities of Spermatozoa in Friesian Holstein Bulls Born in Iraq

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Doi: https://doi.org/10.37940/AJVS.2020.13.2.11

Received: 2/9/2020 Accepted:4/12/2020

Abstract

This study was aimed to evaluate the influence of months of the year on the quality of semen in Holstein bulls. A study carried out at artificial insemination center/ Abou-Ghareeb/ western of Baghdad. A total of 160 ejaculates were collected from 15 bulls born in Iraq via artificial vagina. The age of the bulls ranged between 4 to 5 years and the study period were December to March and September. The semen samples were diluted with Tris base extender. The semen were packed in a straw according to the program of artificial insemination center. Semen characteristics (plasma membrane, acrosome, and DNA integrities) were evaluated. The results revealed a significant decrease ($P \leq 0.01$) in plasma membrane and acrosome integrity in September as compared with December, January, February, and March. There was a significant decline ($P \leq 0.05$) in DNA integrity in September as compared with December, January, February, and March in fresh and frozen semen. In conclusion, the September month had a negative effect on the plasma membrane, acrosome, and DNA percentage in all bulls.

Keywards: Holstein bulls, Semen, DNA, Acrosome, HOST

تأثير التغيرات الشهرية على غشاء البلازما ، الأكروسوم وسلامة المادة الوراثية في نطف ثيران الفريزيان هولشتاين المولودة في العراق

الخلاصة

هدفت هذه الدراسة إلى تقييم تأثير أشهر السنة على جودة السائل المنوي في ثيران الهولشتاين. تم اجراء هذه الدراسة في مركز التلقيح الاصطناعي / أبو غريب/ غرب بغداد. تم جمع 160 قذفة من 15 ثور مولود في العراق باستخدام المهبل الاصطناعي. كانت أعمار الثيران تتراوح بين 4-5 سنوات. اجريت الدراسة خلال الفترة من كانون الاول إلى أذار وأيلول. خفف السائل المنوي بمخفف الترس واعتمد برنامج تجميد السائل المنوي من قبل مركز التلقيح الاصطناعي. قيم السائل المنوي من خلال فحص سلامة كل من غشاء البلازما والاكروسوم والمادة الوراثية. أظهرت النتائج انخفاضًا معنويًا ($P \leq 0.01$) في سلامة غشاء البلازما وسلامة الأكروسوم في أيلول مقارنة بشهر كانون الاول وكانون الثاني وشباط وأذار. كذلك انخفضت سلامة المادة الوراثية أيضًا بشكل ملحوظ ($P \leq 0.05$) في أيلول مقارنة بشهر كانون الاول وكانون الثاني وشباط وأذار في السائل المنوي الطازج والمجمد. وقد استنتج من الدراسة ان لشهر أيلول تأثير سلبي على غشاء البلازما والأكروسوم ونسبة سلامة المادة الوراثية على نطف جميع الثيران.

Introduction

In most domestic animals, the veterinarians and veterinary specialists used artificial insemination (AI)(1). Characteristics of fresh and post-thaw bovine semen are affected by many factors such as age, genotype, breed and individual traits(2-9). However climate, relative humidity, season, daylight and ambient temperature can also affected quality(4,8,10-12). Ball and Peters(13) reports that the ambient temperature affect the characteristics of sperm at collection time, during spermatogenesis and during maturation of spermatozoa in the epididymis, Approximately 70 days prior to collection. It has been reported by several authors that seasonal and monthly changes in semen quality induced by disorders of thermoregulatory mechanisms due to heat stress (11,12,14-18) reducing conception and increasing embryo mortality (19-21). Garcia-Peniche *et al.* (22) observed that Holsteins are sensitive to heat stress which was also reported by several workers during 1968 to 2020 in Iraq (23-27) .

Spermatozoa has a functional membrane needed for the Sperm fertilizing potential, since it is an essential part of sperm capacitation, acrosomal reactions, and attachment of sperm to the surface of the egg (28). The hypoosmotic swelling (HOS) test (29), acrosome integrity (30,31) and DNA integrity (32), it acts as a valuable measure of sperm fertility capacity (33). The current study was aimed to know the effect of months of collection includes Dec. to March and September on fresh and thawed sperm characteristics in Holstein bulls born in Iraq.

Materials and methods

Fresh and thawed semen characteristics from 160 ejaculates were collected from fifteen Holstein bulls by artificial vagina during the period from December, January, February, March and September, presented at the Artificial Insemination Center, Abou-Ghareeb, Iraq. The animals aged between 4 to 5 years. Semen (n = 160) was collected from bulls weekly by artificial vagina method. At collection, fresh semen was kept in water bath at 37Cº diluted with Tris-Fructose-Egg Yolk-Glycerol (TFEG) extender to yield 80 million sperms per ml, cooled to 5 Cº 4hrs packed in 0.25 ml polyvinyl French straws (IMV, France) placed horizontally on a rack in 4cm above liquid nitrogen (LN2) for 9 min then dipped in LN2(34). Frozen straws were melted at 37 Cº 30 seconds in a water bath. For fresh and thawed samples spermatozoa assessment, plasma membrane integrity by HOST as described by Ramu and Jeyendran (28). Sperm acrosomal integrity were calculated using Giemsa stain as described by Kovacs and Foote (35). Sperm DNA integrity by comet assay as described by Hughes *et al.* (36) and Tarozzi *et al.* (37). According to SAS (38), statistical analysis was conducted, followed by the Duncan test to detect important ($p < 0.05$) variations .

Results and discussion

The values of fresh and post-thaw semen characteristics at different months were presented in table (1 and 2). Results revealed a significant decrease (P≤0.01) in plasma membrane integrity in September (51.8±1.64 and 42.2±1.87) as compared with December (62.6±2.36 and 61.4±2.28), January (66.2±2.36 and 56.3±2.35), February (63.3±1.81 and 55.8±2.38) and March (60.1±2.24 and 52.1±3.53) in fresh and frozen semen. This monthly variation might be due to heat stress that affect sperm cells lead to production of reactive oxygen species (ROS), this will affect Poly Unsaturated Fatty Acid (PUFA) on sperm plasma membrane and due to very low antioxidant enzymes in sperm cells makes it susceptible to oxidative stress leads to loses of plasma membrane fluidity that decreased sperm function and motility (39-41).

Table (1): Monthly variation in fresh semen characteristics of Friesian Holstein bulls (Mean ± SE).

Months	Semen parameters (Mean ± S.E)		
	Plasma Membrane integrity %	Acrosome Integrity %	DNA integrity %
December	62.6±2.36 a	86.4±0.97 a	90.0±0.50 a
January	66.2±2.36 a	88.3±1.14 a	88.3±0.88 a
February	63.3±1.81 a	85.0±1.14 a	87.6±1.02 a
March	60.1±2.24 a	82.5±1.70 a	87.8±1.13 a
September	51.8±1.64 b	69.0±1.36 b	78.9±1.34 b
Sig.	0.0075 **	0.0042 **	0.0374 *

Means having with the different letters in same column differed significantly .

*** (P≤0.05), ** (P≤0.01).**

Also, the acrosome integrity decreased significantly (P≤0.01) in September (69.0±1.36 and 59.8±1.72) compared to December (86.4±0.97 and 80.1±0.93), January (88.3±1.14 and 79.0±1.18), February (85.0±1.14 and

76.7±1.31) and March (82.5±1.70 and 73.3±1.88) in fresh and frozen semen, respectively. Al madaly *et al.* (42) suggested that heat stress causes an increase in abnormal spermatozoa and damaged acrosomes .

Furthermore, the DNA integrity also decline significantly (P≤0.05) in September (78.9±1.34 and 75.8±1.24) compared to December (90.0±0.50 and 83.9±0.76), January (88.3±0.88 and 82.1±1.01), February (87.6±1.02 and 82.1±1.09) and March (87.8±1.13 and 82.1±1.41) in fresh and frozen semen, respectively. This variation in DNA damage might be due to heat stress. Borg *et al.* (43) stated that at the time of heat stress, spermatids present within the testis have a higher degree of DNA damages. When the ambient temperature continued to rise may leads to decreased blood flow and the testicular tissues becomes hypoxic lead to extreme production of ROS and the thermoregulatory mechanism disturbed (40,44).

Conclusion

Thus, it was concluded from this study that the September month adversely affect the various bio-physical characteristics of semen in Friesian Holstein bulls. December, January, February and March were the most favorable months for good quality semen production.

Table (2): Monthly variation in post-thaw semen characteristics of Friesian Holstein bulls (Mean ± SE).

Months	Semen parameters (Mean ± S.E)		
	Plasma Membrane integrity %	Acrosome Integrity %	DNA integrity %
December	61.4±2.28 a	80.1±0.93 a	83.9±0.76 a
January	56.3±2.35 ab	79.0±1.18 a	82.1±1.01 ab
February	55.8±2.38 ab	76.7±1.31 ab	82.1±1.09 ab
March	52.1±3.53 b	73.3±1.88 b	82.1±1.41 ab
September	42.2±1.87 c	59.8±1.72 c	75.8±1.24 b
Sig.	0.0068 **	0.0001 **	0.0492*

Means having with the different letters in same column differed significantly .

*** (P≤0.05), ** (P≤0.01).**

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