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SEMINAL AND TESTOSTERONE-HORMONE EVALUATION IN IRAQI-BORN HOLSTEIN FRIESIAN BULLS USED FOR ARTIFICIAL INSEMINATION

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Abstract

The present study aims to evaluate correlation between the testosterone hormone, seminal characteristics and sexual activity in Holstein Friesian bulls that used as the main source of semen production for artificial insemination in Iraq. Totally, 15 adult bulls of 3-4 years old were subjected initial measurement of testosterone serologically using the competitive enzyme-linked immunosorbent assay and for twice-weekly collection of semen to be examined macroscopically and microscopically during October 2019 to March 2020. The findings detected that the concentration of testosterone hormone elevated significantly ($P<0.043$) in high sexuality bulls and reduced in poor sexuality bulls; whereas, time-interval between ejaculates were decreased in high sexuality bulls and increased in poor sexuality bulls. Significantly, the highest value of time-intervals among study bulls were showed in January and the lowest in November ($P<0.036$). Seminal characteristics of high sexuality bulls showed significant increases ($P<0.05$) in values of volume, mass motility, individual motility and live sperms when compared to medium and poor sexuality bulls. For concentration, though there no significant differences ($P>0.05$) were reported between high and medium sexuality bulls, however both groups reported significantly ($P<0.05$) higher values than poor sexuality bulls. Statistically, the highest percentage of dead and abnormal sperms was recorded in poor and medium sexuality bulls; whereas, the lowest were seen in high sexuality bulls. Association of seminal characteristics to month of semen collection revealed on significant improvement in some monthly ejaculates and vice versa.

Key words: Holstein Friesian bulls, Testosterone, Semen, Artificial insemination, Iraq.

Introduction

Artificial insemination is a very common practice in the agriculture world. It involves using collected semen to breed an animal, versus using a live bull to provide the breeding services (Foote, 2010). The semen is kept frozen in straws and then a vet or artificial insemination tech deposits it in the animal at the proper time, depending on their heat (ovulation) cycle (Cseh *et al*., 2012). Holstein Friesian cattle, known as the world's highest-production dairy animals, are a breed of dairy cattle originated in the Netherlands approximately 2,000 years ago. Two breeds of cattle, white animals from the Dutch provinces of North Holland and Friesland and black animals from the Schleswig-Holstein in Northern Germany, were crossed to create a new breed of cattle. This crossbreeding led to a high milk producing animal that was able to do on limited feed sources, originally named as Holstein-Friesian (Ajmone Marsan *et al*., 2010; Danchin-Burge *et al*., 2011). After that, this breed is raised in Scandinavian countries (Philipsson and Lindhé, 2003), Britain (Albarrán-Portillo and Pollott, 2013), North America (Djemali and Berger, 1992), Africa (Kim and Rothschild, 2014) and Asia particularly Middle East due to its adaptability to hot weather and high production (Ajmone-Marsan *et al*., 2010). In addition, the animal of this breed is calm, easily managed and the male can be raised for beef production with a good dressing percentage (Kadokawa and Martin, 2006). Morphologically, Holstein Friesian characterized by its black and white or white and red colors and that the red color is a recessive and undesirable character (Robinson *et al*., 1993). Among cattle livestock, this breed is considered as the largest dairy one with an average weight of 600-650 kg, 800-850 kg and 35-45 kg for cow, bull and calf at birth, respectively. Regarding to milk yield,

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Holstein Friesian cow can produce approximately 5000kg within 305 days of production with 3.5-3.8% of butter fat that comparatively is low due to a large quantity of milk production (Shamiah *et al*., 2007; Kuczyñska *et al*., 2011). For a purpose of improving the productivity of the local cattle livestock, Holstein Friesian breed that introduced to Iraq in 1950, reporting 2500-3000kg an average quantity of milk production during 305 days (Kassir *et al*., 1969).

Bull fertility plays a very important role in bovine reproduction since selection intensity of bulls chosen for breeding programs in both beef and dairy industry is high and since they can produce high numbers of offspring relative to females (Han and Peñagaricano, 2016). Bulls that are sub-fertile lead to prolong calving season due to delay pregnancy, reducing calf crop weights and in turn increasing culling of females. Therefore, problems created by sub-fertile or infertile bulls are of great economic importance to the cow-calf producer (Carpenter and Sprott, 2007). There are several different factors influence on reproductive performance of bulls such as testicular development, seminal quality, libido, mating ability and physical soundness (Smith *et al*., 1981; Singh *et al*., 2018). However, reproductive capability of the bulls is often difficult to assess, because successful reproduction requires the integration of several biologic systems (Petherick, 2005). The evaluation of mature male for breeding soundness requires careful examination of not just the ejaculate, but the entire animal (Chacon *et al*., 1999). Seminal quality is a good predictor of the male fertility and predicting the results of semen analysis is very useful in early diagnosis of seminal disorders, selection of semen donor candidates and prioritization of further infertility treatment (Gil *et al*., 2012; Girela *et al*., 2013; Wang *et al*., 2014). Hence, this study aims to assessment the levels of testosterone hormone among study bulls that used as the main source of semen production for artificial insemination in Iraq, As well as, estimation the efficiency of fresh semen macroscopically and microscopically. Also, relationship between seminal parameters and period of sample collection is targeting in current study.

Materials and Methods

Study animals

This study was carried out at the Artificial Insemination Center in Abu Ghraib, located just the west of Baghdad's city center in Baghdad province, Iraq, from October 2019 until March 2020 on a total 15 sexually adult Iraqi-born Holstein Friesian bulls that have been used as the only source of semen production for artificial insemination straws in Iraq. Age of all study bulls is ranged 3-4 years and all animals the Artificial Insemination Center are existed under identical environmental and management factors and are received a high health care.

Samples collection

• Blood: An overall 5ml of jugular venous blood were drained from each study bull under aseptic conditions using a disposable syringe into a free anticoagulant tube. All blood samples were centrifuged at 3000rpm for 5 minutes and the sera were kept into labeled 1.5 ml eppendorf tubes at -4°C until be used for measuring of testosterone hormone.

• Semen: As a routinely work in the Artificial Insemination Center, the samples semen is collected twice in a week using an artificial vagina. Immediately, all ejaculates were brought to the laboratory of the Artificial Insemination Center to ensure their quality through evaluation of the semen parameters macroscopically and microscopically and subsequently subjected for dilution and straw packaging to be cooled and frozen.

Testosterone measurement

According to manufacturer's instruction (Elabscience, USA), the kit of competitive enzyme-linked immunosorbent assay (ELISA) was used to measurement of testosterone hormone in sera of study bulls. The principle of this kit involves the competing reaction between the testosterone that coating the microplate and the testosterone in the sample or Stander for sites on the Biotinylated Detection Ab specific to testosterone. The procedure of analysis was include the initial pre-warming, preparation and dilution of all reagents and Standard and then, the Standard and sera were loaded to the wells of microplate and incubated (37°C / 45min). Excess conjugate and unbound samples or Standers were washed three times from the plate and Horseradish Peroxidase (HRP) was added, incubated (37°C / 30min) and washed three times. TMB-Substrate Solution was added to each well and incubated (37°C / 15 min). The Enzyme-Substrate reaction was terminated by addition of the Stop-

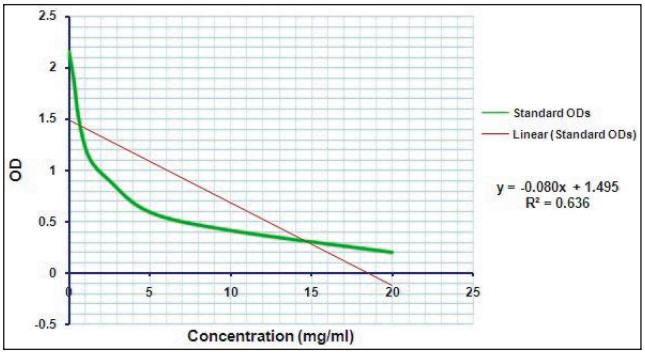

Fig. 1: Reference Standard Curve.

Table 1: Reference Standard Concentrations and their ODs.

Concentration (ng/ml)	OD (nm)
20	0.199
10	0.413
5	0.592
2.5	0.901
1.25	1.126
0.62	1.491
0.31	1.874
0	2.158

Solution and incubation (37°C / 10min) in a dark place to avoid over-reaction. The optical density (OD) value for the wells of Standard and sera was measured at a wavelength of 450nm by the microplate ELISA reader (BioTeK, USA). The concentration of testosterone in the sera was determined by comparing the ODs of the samples to the Standard Curve (Table 1, Fig. 1).

Macroscopic and microscopic evaluation of semen

• Volume: The volume for each ejaculate of each study bulls was recorded directly through a graduated tube of collected semen (Henkel, 2012).

• Mass Motility: A drop of fresh semen was applied on a warm slide, covered and examined microscopically at 10× magnification. The scores of mass motility were classified as following (Table 2), (Fiaz *et al.,* 2010).

• Individual motility: It was recorded by mixing a drop of fresh semen with two drops of sodium citrate solution on a warm slide at a 37°C, to be covered and examined microscopically at a 40× magnification. The scores of individual motility were classified as following (Table 3), (Kharche *et al*., 2013).

• Live and Dead sperms: According to manufacturer instruction (RAL Diagnostics, France), Eosin and Nigrosin stain was used to calculate the percentage of live and dead sperms under 40× magnification of the bright field microscope.

• Sperm abnormality: It was performed using the slide prepared previously for counting of live and dead sperm, but using an immersion oil of the light microscope under a 100× objective lens. Any defects in head and/or tail of the sperm are considered abnormality. The percentage of abnormality was recorded after counting at least of 200 sperms (Freneau *et al*., 2010).

Table 2: Score for classification of mass motility in semen of study bulls

Characteristic	(%)
No wave, total immobility	0
No wave, individual movement	10
No wave, very slow movement	20-40
Wave appearance, slow amplitude of wave	45-65
Wave appearance, rapid wave motion, no eddies	70-85
Wave appearance, rapid wave motion with eddies	90-100

Table 3: Score for classification of mass motility in semen of study bulls.

Characteristic	(%)
No motile sperms	0
1/5 of sperms are motile	10
2/5 of sperms are motile	20-40
3/5 of sperms are motile	45-65
4/5 of sperms are motile	70-85
5/5 of sperms are motile	90-100

Statistical analysis

All data were introduced, arranged and analyzed using the computerized programs; Microsoft Office Word and Excel, Paint and SPSS. One-Way Analysis of Variance (ANOVA) was applied to detect statistical association between the serological results of testosterone hormone of study bulls, macroscopic and microscopic seminal findings and relationship to period of semen collection. Differences in compared findings were considered significance at a level of $P<0.05$ (George and Mallery, 1999; Neyeloff *et al*., 2012).

Results

Testosterone hormone

Based on their sexuality, the Artificial Insemination Center of Iraq divided the study bulls into high, medium and low activity bulls. Hormonal measurement revealed on significant elevation ($P<0.043$) in values of

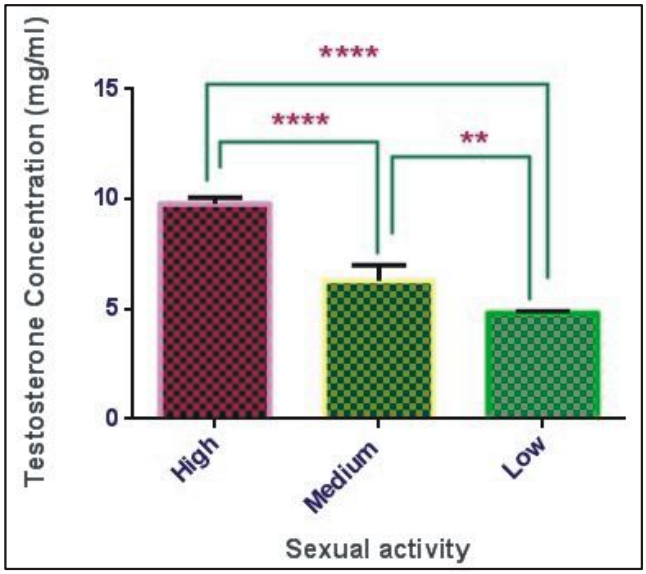

Fig. 2: Concentration of testosterone hormone according to sexual activity. [(**) and (****) referred to significant differences between groups as $p<0.01$ and $p<0.0001$ respectively].

Table 4: Concentration of testosterone hormone regarding to sexual activity of study bulls.

Parameter	Total No.	Sexual activity			*P value*
		High (No: 5)	Medium (No: 5)	Poor (No: 5)	
Testosterone (ng/ml)	15	9.78 ± 0.29^{A} (9.3-10)	6.28 ± 0.73^{B} (5.1-7.1)	4.8 ± 0.1^{C} (4.7-4.9)	P<0.043
Value: M ± SD (R), variation in horizontal large letters referred to significant differences (P<0.05) between groups					

Table 5: Time-interval regarding to sexual activity of study bulls.

Parameter	Total No.	Sexual activity			*P value*
		High (No: 5)	Medium (No: 5)	Poor (No: 5)	
Time-interval (Second)	15	42.16 ± 3.21 (18 - 74)	68.93 ± 4.13 (41 - 98)	91.11 ± 5.23 (64 - 112)	P<0.036
Value: M ± SD (R), variation in horizontal large letters referred to significant differences (P<0.05) between groups					

testosterone hormone among the high sexuality bulls (9.78 ± 0.17) in comparison to medium (6.28 ± 0.42) and poor (4.77 ± 0.07) sexuality bulls respectively (Table 4, Fig. 2).

Time-interval of ejaculates

• Relation to sexual activity: Among groups of study bulls, the high sexuality bulls showed the lowest time-interval required to ejaculate (42.16 ± 3.21); whilst, bulls of poor sexuality were recorded the longest time-interval required to ejaculate (91.11 ± 5.23), at a significance value of P<0.036 (Table 5).

• Relation to month of semen collection and sexual activity: Concerning to month of semen collection among groups of study bulls, the highest and lowest time-interval required for ejaculation were seen in January and November of high (51.26 ± 3.02 and 32.65 ± 2.98), medium (79.31 ± 3.54 and 58.20 ± 4.27) and poor (98.72 ± 4.61 and 81.75 ± 5.01) sexuality bulls (Table 6).

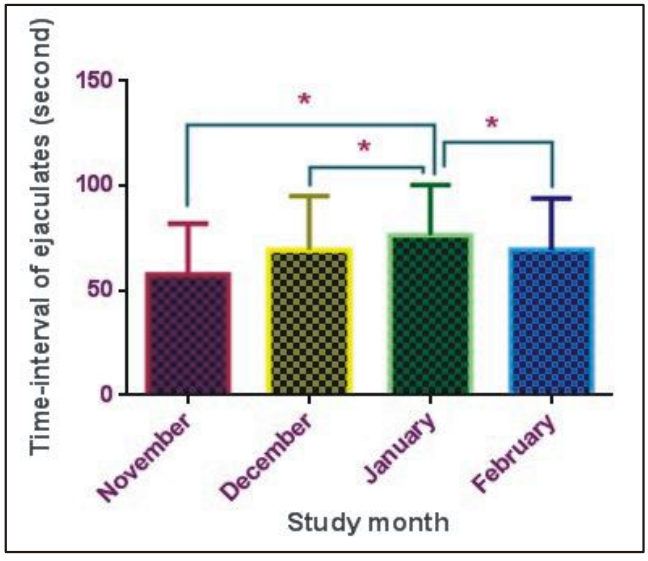

Fig. 3: Concentration of testosterone hormone in relation to month of semen collection. [(*) referred to significant differences between groups as p<0.05].

• Relation to month of semen collection: Statistically, the highest significant (P<0.05) values for time-intervals required for ejaculation were detected in values of January [76.43 ± 13.78 (51.26-98.72)]; whereas, there no significant differences (P>0.05) were showed between values of other study months; November [57.53 ± 14.18 (32.65-81.75)], December [69.19 ± 14.92 (40.61-90.93)] and February [69.44 ± 14.14 (44.12-93.02)], respectively (Fig. 3).

Association of seminal findings to bull's sexuality

Seminal characteristics of high sexuality bulls were showed significant increases (P<0.05) in volume, mass motility, individual motility and live sperms when compared to medium and poor sexuality bulls. For concentration, though there no significant differences (P>0.05) were reported between high and medium sexuality bulls, both groups were significantly (P<0.05) higher than poor sexuality bulls. Statistically, the highest percentage of dead and abnormal sperms was recorded in poor and medium sexuality bulls; whereas, the lowest were seen in high sexuality bulls (Table 7).

Association between month, seminal parameters and sexual activity

• Volume: Among three study groups, the highest significant (P<0.05) value for volume of collected semen was reported in January (5.98 ± 0.17) and December (5.85 ± 0.18) for high sexuality bulls and during November (5.53 ± 0.3 and 4.43 ± 0.29) in medium and poor sexuality bulls, respectively (Table 8).

• Concentration: Significantly, the highest value

Table 6: Time-interval regarding to month of semen collection.

Month	Sexual activity		
	High	Medium	Poor
November	32.65 ± 2.98^{Cc} (18 - 46)	58.20 ± 4.27^{Bc} (49-87)	81.75 ± 5.01^{Ad} (66-97)
December	40.61 ± 3.55^{Cb} (28 - 51)	67.02 ± 3.90^{Bb} (41 - 83)	90.93 ± 6.28^{Ac} (64 - 103)
January	51.26 ± 3.02^{Ca} (38-74)	79.31 ± 3.54^{Ba} (53-98)	98.72 ± 4.61^{Aa} (75-112)
February	44.12 ± 3.29^{Cb} (23-67)	71.18 ± 4.82^{Bb} (51-95)	93.02 ± 5.03^{Ab} (64-108)
Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R), Unit: Second			

Table 7: Results of seminal parameters among different sexual activity study bulls.

Parameter	Unit	Sexual activity		
		High	Medium	Poor
Volume	ml	5.54 ± 0.25^{A}(4.8 - 6.3)	4.94 ± 0.23^{B}(3.8 - 6.1)	4.07 ± 0.19^{C}(3.2 - 5.1)
Concentration	10^9/ml	1.73 ± 0.15^{A}(1.19 - 2.38)	1.37 ± 0.09^{A}(0.87- 1.69)	1.12 ± 0.17^{B}(0.52 - 1.39)
Mass motility	%	58.12 ± 3.1^{A}(50 - 65)	49.69 ± 6.37^{B}(30 - 65)	43.19 ± 5.87^{C}(25 - 60)
Individual motility	%	66.29 ± 3.98^{A}(55 - 80)	58.13 ± 6.34^{B}(35 - 75)	52.81 ± 5.71^{C}(35 - 70)
Live sperms	%	85.29 ± 1.54^{A}(65 - 91)	71.55 ± 2.02^{B}(60 - 85)	66.14 ± 3.1^{C}(55 - 80)
Dead sperms	%	15.13 ± 1.54^{C}(9 - 35)	28.63 ± 1.84^{B}(15 - 40)	33.77 ± 1.37^{A}(20 - 45)
Abnormal sperms	%	12.51 ± 1.32^{C}(5 - 25)	27.33 ± 1.89^{A}(11 - 40)	22.02 ± 1.36^{B}(13 - 36)
Large horizontal letters refer to significant differences (P<0.05), Value: M ± SD (R)				

Table 8: Seminal volume of different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	5.23 ± 0.5^{Ab} (5.1-5.5)	5.53 ± 0.3^{Aa} (4.7-6.1)	4.43 ± 0.29^{Ba} (3.8-5.1)
December	5.85 ± 0.18^{Aa} (5.4-6.2)	5.01 ± 0.14^{Bb} (4.6-5.3)	3.78 ± 0.2^{Cb} (3.2-4.1)
January	5.98 ± 0.17^{Aa} (5.6-6.3)	4.43 ± 0.31^{Bc} (3.8-5.1)	4.13 ± 0.1^{Ba} (3.9-4.3)
February	5.1 ± 0.13^{Ab} (4.8-5.4)	4.78 ± 0.18^{Bb} (4.3-5.2)	3.95 ± 0.15^{Ca} (3.7-4.3)
Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)			

Table 9: Seminal concentration of different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	1.61 ± 0.18^{Ac} (1.22-2.31)	1.29 ± 0.09^{Bb} (0.96-1.43)	0.98 ± 0.18^{Cd} (0.52-1.15)
December	1.74 ± 0.13^{Ab} (1.31-2.09)	1.33 ± 0.12^{Bb} (0.87-1.48)	1.09 ± 0.16^{Cc} (0.69-1.24)
January	1.71 ± 0.19^{Ab} (1.19-2.38)	1.41 ± 0.09^{Ba} (1.09-1.61)	1.15 ± 0.17^{Cb} (0.74-1.31)
February	1.85 ± 0.09^{Aa} (1.42-2.26)	1.45 ± 0.07^{Ba} (1.20-1.69)	1.25 ± 0.18^{Ca} (0.78-1.39)
Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)			

(P<0.05) for concentration of ejaculate sperms was recorded in February (1.85 ± 0.09), February (1.45 ± 0.07) and January (1.41 ± 0.09) and February (1.25 ± 0.18) for high, medium and poor sexuality bulls, respectively (Table 9).

• Mass motility: During February (62.5 ± 2.5) and December (58.75 ± 4.27), there are significant increases (P<0.05) in mass motility of high sexuality bulls and in February for medium (58.75 ± 6.25) and poor (50 ± 6.77) sexuality bulls (Table 10).

• Individual motility: In high sexuality bulls, significant

Table 10: Seminal concentration of different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	53.72 ± 2.39^{Ab} (50-65)	41.25 ± 7.18^{Bd} (30-60)	38.75 ± 5.54^{Bb} (25-50)
December	58.75 ± 4.27^{Aa} (50-65)	46.25 ± 4.27^{Bc} (35-55)	41.25 ± 6.88^{Cb} (25-55)
January	57.5 ± 3.23^{Ab} (50-65)	52.5 ± 7.77^{Bb} (30-65)	42.75 ± 4.27^{Cb} (35-55)
February	62.5 ± 2.5^{Aa} (50-65)	58.75 ± 6.25^{Aa} (40-65)	50 ± 6.77^{Ba} (30-60)
Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)			

Table 11: Individual motility of semen among different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	60 ± 3.54^{Ab} (55-70)	51.25 ± 8.03^{Bc} (35-65)	48.75 ± 5.54^{Cc} (35-60)
December	68.75 ± 3.15^{Aa} (55-75)	55 ± 6.12^{Bc} (40-65)	50 ± 6.45^{Cb} (35-65)
January	66.25 ± 4.27^{Aa} (55-75)	60 ± 6.12^{Bb} (45-70)	53.75 ± 5.54^{Cb} (40-65)
February	70.16 ± 5.04^{Aa} (55-80)	66.25 ± 5.15^{Aa} (55-75)	58.75 ± 5.54^{Ba} (45-70)
Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)			

increases (P<0.05) in individual motility of semen were seen in February (70.16 ± 5.04), December (68.75 ± 3.15) and January (66.25 ± 4.27), respectively. For medium and poor sexuality bulls, the highest value of individual motility was detected in February, 66.25 ± 5.15 and 58.75 ± 5.54, respectively (Table 11).

• Live sperms: The highest significant increases (P<0.05) in values of live sperms were reported in February (89.10 ± 0.98) and January (87.29 ± 1.36) among high sexuality bulls; November (74.59 ± 2.81), February (73.51 ± 2.10) and December (70.16 ± 1.80)

Table 12: Percentage of live sperms among different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	81.05 ± 2.16 ^{Ab} (65-85)	74.59 ± 2.81 ^{Ba} (60-85)	68.31 ± 2.57 ^{Ca} (60-75)
December	83.71 ± 1.64 ^{Ab} (76-88)	70.16 ± 1.80 ^{Ba} (65-79)	63.10 ± 3.25 ^{Cb} (55-75)
January	87.29 ± 1.36 ^{Aa} (77-90)	67.92 ± 1.37 ^{Bb} (60-77)	64.28 ± 2.89 ^{Ba} (60-72)
February	89.10 ± 0.98 ^{Aa} (83-91)	73.51 ± 2.10 ^{Ba} (65-80)	68.85 ± 3.67 ^{Ba} (60-80)

Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)

Table 13: Percentage of dead sperms among different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	19.68 ± 1.82 ^{Ca} (15-35)	25.51 ± 2.24 ^{Bb} (15-40)	31.62 ± 1.28 ^{Aa} (25-40)
December	15.94 ± 1.54 ^{Ca} (12-24)	29.68 ± 2.06 ^{Ba} (21-35)	36.85 ± 1.49 ^{Aa} (25-45)
January	13.03 ± 1.62 ^{Bb} (10-23)	31.90 ± 1.68 ^{Aa} (23-40)	35.59 ± 1.12 ^{Aa} (28-40)
February	11.86 ± 1.17 ^{Bb} (9-19)	27.44 ± 1.39 ^{Aa} (20-35)	31.01 ± 1.60 ^{Aa} (20-40)

Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)

Table 14: Percentage of abnormal sperms among different sexuality bulls during months of study.

Month	Sexual activity		
	High	Medium	Poor
November	16.30 ± 1.56 ^{Ba} (10-25)	24.88 ± 2.37 ^{Aa} (11-36)	23.09 ± 1.31 ^{Aa} (20-31)
December	14.64 ± 1.15 ^{Ba} (10-21)	29.13 ±1.63 ^{Aa} (20-35)	27.92 ± 1.56 ^{Aa} (22-36)
January	10.17 ± 1.31 ^{Cb} (5-19)	28.96 ± 1.84 ^{Aa} (21-40)	22.80 ± 1.25 ^{Bb} (19-32)
February	8.93 ± 1.24 ^{Cb} (5-18)	26.34 ± 1.75 ^{Aa} (16-34)	18.26 ± 1.30 ^{Bb} (13-26)

Large horizontal and small vertical letters refer to significant differences (P<0.05), Value: M ± SD (R)

for medium sexuality bulls; and in February (68.85 ± 3.67), November (68.31 ± 2.57) and January (64.28 ± 2.89) for poor sexuality bulls (Table 12).

• Dead sperms: Significantly high (P<0.05) percentage of dead sperms were observed during November (19.68 ± 1.82) and December (15.94 ± 1.54) for high sexuality bulls and in January (31.90 ± 1.68), December (29.68 ± 2.06) and February (27.44 ± 1.39) for medium sexuality bulls. However, there no significant differences (P>0.05) were recorded between the values of all study months of poor sexuality bulls (Table 13).

• Abnormal sperms: Although, the medium sexuality bulls were showed no significant variation (P>0.05) in percentage of abnormal sperms among all study months, significant elevation (P<0.05) was detected in November (16.30 ± 1.56) and December (14.64 ± 1.15) for high sexuality bulls and December (27.92 ± 1.56) and November (23.09 ± 1.31), respectively for the poor bulls of sexual activity (Table 14).

Discussion

The ability to predict the fertility of bulls before semen is released into the field has been a long-term objective of the animal breeding industry and the recent shift in the industry towards the intensive use of genomically selected bulls has increased its urgency (Fair and Lonergan, 2018). In this study, high sexuality bulls were showed a significant elevation in concentration of testosterone hormone that decreased in medium and more severely in poor sexuality bulls. These findings are agreed with Aksoy *et al*., (2002) and Chacur *et al*., (2013), but are incompatible to that detected previously (Foote *et al*., 1976; Mahmood *et al*., 2013) as there is no association between sexual activity and testosterone. Hafez and Hafez, (2013) reported that the concentration of circulating testosterone in Holstein Friesian bulls is unrelated to sexual activity or semen characteristics. However, Foote *et al*., (1976) demonstrated that there was a tendency for testosterone to increase with age in Holstein bulls up to 6-7 years. Widyaningrum *et al*., (2015) detected that the group housing management of young bulls resulting in puberty acceleration through increasing of testosterone hormone; and poor quality handling can cause low libido, sperm quality and triggers. As the study bulls were young and received similar management and environmental conditions, we thought that the concentration of other hormones such as GnRh, LH and estrogen might influence on the testosterone secretion as detected previously (Chacur *et al*., 2013; Monaco *et al*., 2015). In addition, a recent study focusing on the epigenetic profiles of young bulls highlighted that young bulls have a different sperm DNA methylation pattern at each specified age group (Lambert *et al*., 2018).

Concerning to time-interval of ejaculates, we found that high sexuality bulls have a significant lower values in comparison to poor sexuality bulls that appeared with a longer period. Also, the time was decreased significantly in November and increased in January among all study bulls of different sexuality. Kommisrud and Berg, (1996)

reported that time of sexual preparation have significant effects on ejaculation profile (ejaculate volume, number of doses per ejaculate and post-thaw motility). Previously, beef and dairy producers have suggested that the sex ratio of the calf crop varied among bulls. Chandler *et al*., (1998, 2002) demonstrated that there variations in the percentage of Y chromosome-bearing spermatozoa (%Y-CBS) between ejaculates within the same bulls are found. Singh *et al*., (2015) showed that the poor libido bulls are usually take a higher total time in mount that may or may not be resulted in successful ejaculation. Mahmood *et al*., (2013) mentioned that testosterone could be affected the ability of males for erection and ejaculation, as well as on the quality and quantity of semen. However, many studies were detected that the ejaculate volume, sperm concentration and total number of spermatozoa per ejaculate can increase with increasing collection interval and suggested that the optimum intervals of 3-5 days for percentage of motile sperm (Igna *et al*., 2010; Murphy *et al*., 2018). In relation to seasonal influences, study bulls of different sexual activity were found to be almost same since significant increase in time-interval were showed in January. This might be attributed to increase environmental stressful condition during this season, winter. Worldwide, many studies have been estimated the effect of season on time-interval and sexual activity with conflicting results (Ahmad *et al*., 2005; Farooq *et al*., 2013; Mahmood *et al*., 2013). Locally, AL-Badry *et al*., (2012) indicated that best sexual desire was in February and best some semen parameters were in January and February.

Regarding to seminal profiles, the findings of our study showed that there are significant increases in seminal quality among high sexuality bulls when compared to medium and poor sexuality bulls. Several studies have analyzed the relationship between serum testosterone concentration, sperm quality and fertility in bulls with a few reporting a strong association (Javed *et al*., 2000; de Brito, 2006; Rajak *et al*., 2014). These reports were in agreement with that detected in this study as the high sexuality bulls have a high serum testosterone concentration as well as a high seminal quality. Based on previous reports, the findings showed that the optimum testosterone concentration with larger sized scrotal circumference and seminal vesicles plays an important role in enhancing the bull productivity of spermatozoa per gram testicular tissue (Almquist and Amann, 1961) and semen quality (Pant *et al*., 2003). Moreover, relations across gender suggested that there certain genes may affect some critical reproductive mechanisms in both males and females such as reproductive organ size (testis and seminal vesicles) that is an indicator of fertility and marker of the timing of puberty (Argyropoulos and Shire, 1989; Foote, 2003). Mahmood *et al*., (2018) concluded that there is a significant positive correlation between scrotal circumference and fertility parameters related to seminal vesicles weight and its secretion: fructose, citric acid, protein and testosterone and suggested that this approach along with certain semen quality attributes reflecting epididymis function could be used as a predictive fertility marker for grading and selection of breeding bulls and their progenies to develop outstanding bull mother farm.

Association of seminal parameters to month of semen collection in present study was revealed a significant variation in their findings. The effect of season on bovine semen production has been widely assessed but with conflicting data perhaps due to the range of climatic conditions under which these studies have been carried out (Gadea, 2005). However, many studies reported that seasonal variation in semen characteristics have mainly attributed these changes to compromised scrotal thermoregulation and heat dissipation mechanisms, as well as the endocrine profile and differential response of bulls testes to gonadotropins (Jiménez-Severiano *et al*., 2003; Menegassi *et al*., 2011). Seasonal variation associated with photoperiod, in particular luteinizing hormone (Chandolia *et al*., 1997), testosterone concentration (Souza *et al*., 2011) and melatonin levels (Li *et al*., 2019), can affect spermatogenesis. Moreover, the adaptability of a bull to local microclimatic conditions may have consequences for semen quality and therefore, could account for differences in their reproductive capacity throughout the year (Brito *et al*., 2002, Herbut *et al*., 2018). Other reports observed that increase in seminal quality and quantity is associated with increasing bull age and increase in sperm motility and viability is driven increases in ejaculate volume up to 4 years of age (Bhakat *et al*., 2011; Baliæ *et al*., 2012). Murphy *et al*., (2018) reported a correlation between season, bull age and seminal characteristics since there was a tendency for summer and autumn to increase ejaculate volume, sperm concentration, total sperm number and gross motility. Therefore, increasing in ejaculate volume with age may be related to an increase in activity of the hypothalamic-pituitary-testicular axis and the concurrent development of the testis and accessory glands with sexual maturity, which believed to continue to develop for up to 5 years post-puberty (Almquist, 1978; Murphy *et al*., 2018).

Conclusion

Our findings confirmed that the concentration of testosterone hormone in addition to macroscopic and microscopic seminal characteristics correlate positively with the sexual activity of Holstein Friesian bulls. Also, there is relationship between

Contribution of Authors

Jaafar J.A. Al-Chaabawi and Hayder A. Hasan were responsible for blood sampling, measurement of testosterone hormone and assessment of some seminal parameters; whilst, Khitam J.Y. Alazawe and Faris F.I. Mohammed were responsible for collection of semen and evaluation of some semen parameters. All authors read and approved the final manuscript.

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