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Individual Variation in Semen Attributes and Number of Straws Produced by Holstein Bulls Born in Iraq During Different Ages and Months

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ABSTRACT

THIS study aimed to determine the individual differences among the Holstein bulls born in Iraq for fresh and cryopreserved semen. Moreover, the effect of age (2,3,4 and 5 years) and months (January, February, March, April, and November) on fresh and cryopreserved semen quality was also studied. The study was conducted at the Artificial Insemination Department of the Ministry of Agriculture, Iraq. Data of eligible fresh semen were the ejaculate volume and mass activity percentage, whereas for cryopreserved semen were dilution rate, post-diluted, post-cooling, and post-thawed individual motility percentage and number of straws produced from thirteen Holstein bulls aged 2-6 years that used for semen production in above mentioned months of 2022. Results showed that variations were observed in fresh (ejaculate volume, mass activity percentage) and frozen semen production (dilution rate, individual motility percentage after dilution, cooling, and post-thawing, and a number of straws)were significant among individual Holstein bulls born in Iraq for 3 and 5 years which the analysis here still applies for artificial insemination. Moreover, some bulls at the age of 3 years showed the best semen quality and frozen semen production in January, February, and November, and others in March and April, while bulls were at five years old in January, February, and April, and another in March and November. Also, the current results revealed that the month and age had a significant effect on the semen properties and the number of straws produced, as it was found that the best traits at age 3-4 years compared to age 2 and 5 years and that the month of March and April is better than the months January, February, and November. **Conclusion:** This study shows an obvious variation in semen quality resulting from the individual differences among Holstein bulls born in Iraq, and bulls aged 3-4 years produced the best semen properties and number of straws. Moreover, the highest characteristics only in the months of March and April.

Keywords: individual variation, Holstein bulls, frozen straws.

Introduction

One of the most effective and important biotechnological approaches is artificial insemination (AI). It enables breeders of dairy cattle to use quality validated AI sires and thus boost their genetic capacity and increase the productivity of their herds [1]. The artificial insemination center plays an significant role in providing high-quality semen, from the fresh semen collection to the frozen semen quality assessment process, which will influence the effectiveness of artificial insemination across both of these operations. In the selection and rearing of animals, the artificial insemination center also has a significant role in making optimum performance. Selecting superior bulls requires consideration of several factors. The quality of bull semen can be affected by a number of factors including, genetic characteristics, season, age of bull, frequency of ejaculation and nutrition [2,3].

Unless the sperm comes from high genomatic bulls, the diffusion of their sperm is equal to the best genetic material selected, however, the standard of semen needs to be considered and continually assessed to produce successful results in the breeding programme, the consistency of semen therefore has a strong correlation with fertility [4, 5]. Hence ensuring high-quality semen will sustain and boost the performance and efficiency of AI applications. In the animal breeding system and industry, the sperm production and quality are among factors that should be

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considered. Several factors, such as temperature, have reportedly affected fresh semen quality in fresh state, genetic**,** season , individual variation**,** age and interval collection [6]. A broad range of environmental and genetic factors, including bull age, collectivity period, collection frequency and season, can affect semen quality [7].

It is well known that the age of a bull at the time of collection affects semen attributes such as sperm output and quality [8]. Older bulls have more volume and consistency of semen than younger bulls[9]. This rise is mainly attributed to physiological changes including an increase in body weight[10]. simultaneous growth of post-puberty testis and accessory glands and sexually matured, leading to an rise in semen production. [11]. The aim of the present study were to evaluates the individual differences in some properties of semen and the number of straws produced by Holstein bulls born in Iraq and the relationship of those differences with age and monthly changes.

Material and Methods

Our study was done in the center of artificial insemination, west of Baghdad on Holstein bulls at the age three years (3Bull) and four years (5 Bull). The semen were routinely collected weekly from all bulls using artificial vagina. During the study period beginning from January 2022 until December 2022, all bulls were maintained on equal terms of management, feeding and watering. Ejaculates were taken weekly from each bull by using the artificial vagina method. 2-3 false mounting is performed in order to sexually stimulate the bull to maximize semen collection [12]. 135 ejaculates were studied during the period of this experiment. As a routine work in the AI center, The sample was subsequently brought into the laboratory and placed in a water bath following semen collection at (37-38˚C) for macroscopically evaluation volume of semen (ml) and microscopic evaluation mass activity % , individual motility % for both after dilution, after cooling and post-thawing.

Statistical analysis. The effect of month of collection, age of bull and number of straws on Semen properties were analyzed by two-way ANOVA with the general linear model method described [13]. Comparisons between values were analyzed by Duncan's multiple range test following an F-test in ANOVA [14]. Significance was set at ($P<0.05$).

Results

1- Individual variation in semen properties and number of straws for age 3 year of Holstein bulls:

The effect of the individual bulls on the quality traits evaluated after collection (volume in ml, mass motility%) and steps of freezing (dilution rate, individual motility% of sperms after dilution and cooling post-thawing) and calculated number of straws during January, February, March, April and November months for are presented in (Table 1).

1-During January : The volume of ejaculate in bulls 1711(7.77ml) and 1827(7.14ml) different significant ($P<0.05$) compare with bull 1810 (3.70ml) and differences significant ($P<0.05$) among sire1711 with 1810 and 1827 in mass motility% (48.88,42.85,43.33), dilution rate (23.33,15.57,19.37) and number of straws (313.75,455.71,304.41) sequentially, moreover different significant ($P<0.05$) between 1827 compare with 1810 and 1711 bulls in individual motility% of sperms after cooling (54.28,58.75,60.55), post- thawing (50.01,54.16,55.55) and number of straws (455.71,304.41,,796.88) alternately but the another's comparative no significant such as between 1711 and 1827 in volume ejaculate, 1711 with 1810, and between 1827 with 1810 in individual motility% of sperms after dilution, also the different no significant between 1711 and 1810 in individual motility% of sperms for all steps of freezing.

2-During February : The volume of ejaculate (ml), mass motility%, individual motility % of sperms after dilution, cooling ,dilution rate and numbers of straws in bulls 1711(10.23ml,50,54,65.54,60.12,25.43,722.54) different significant ($P<0.05$) compare with bull 1827 (5.57ml,37.14,55.01,49.28,9.14,220.66) and 1810 (4.20,40.01,57.06,51.04,18.20,313.75) respectively and differences significant ($P<0.05$) in dilution rate among sire 1810 (18.20) with 1827 (9.14), but the another's comparative no significant such as between 1810 and 1827 in volume ejaculate, mass motility %,individual motility % of sperms (after dilution, cooling) and numbers of straws, in addition the different no significant between sir 1711 with 1810 in individual motility% of sperms for post-thawing.

3-During March: Result in Table 1revealed that the differences significant ($P<0.05$) in mass motility% of sperms for all bulls 1711 (50.33),1810 (41.66) and 1827 (33.34) ,and high significant ($P<0.05$) in dilution rate and individual motility % of sperms after dilution and post thawing for bulls 1711(15.67,55.64,50.32) and 1810 (16.33,60.83,52.50) among bull 1827 (10.90,47.27,39.45),moreover number of straws production high significant ($P<0.05$) by bulls 1810 (349.36) and 1827 (352.25) compare with 1711 (232.54) ,in another hand less significant ($P<0.05$) in individual motility % of sperms after cooling for 1827 (47.77) compare with 1810 bulls (56.25) which the differences in this parameter no

significant between bull 1711(52.54) compare with each bulls 1810 and 1827 , and also the differences no significant between all bulls in volume of semen in this month , moreover the different no significant between bulls 1711 and 1810 in dilution rate and individual motility % of sperms for post – thawing, also no different significant among bulls 1810 and 1827 (Table 1) .

4-During April: Present study show that mass motility% , dilution rate, individual motility% of sperms for post- thawing and number of straws for bull 1810 (45.55,18.88,56.11,434.01) registered highest level significant (P<0.05) than bulls 1827(37.14,8.57,51.42,349.17) in addition to individual motility% of sperms after dilution and cooling for bulls 1810 (65.03,60.55)and 1827 (62.94,57.14) registered more significant (P<0.05) than bull 1711(57.06,50.34) but volume of ejaculate for bull 1827(7.57) highest significant (P<0.05) than both bulls 1810 (4.27) and 1711(5.03).but the different no significant between bulls 1810 and 1711 in volume of semen, mass motility% of sperms and dilution rate , also the different no significant between bulls 1810 and 1827 in individual motility% of sperms after dilution and cooling , in addition to the different no significant between bulls 1711 and 1827 in mass motility and individual motility% of sperms for post-thawing.

4-During November: A statistically significant difference (P<0.05) was found in dilution rate and number of straws of sires 1711(14.60, 762.66), 1810 (22.60, 495.50) and 1827 (9.41, 290.25) as well as volume of ejaculate of bull 1711(7.41) high significant (P<0.05) than 1827 (5.40) and 1810 (4.28) but individual motility % of sperms after dilution for bull 1827 (61.12) more significant (P<0.05) than bulls 1810 (55.02) and 1711(53.33), results in Table 1 show that the others differences no significant in mass motility % and individual motility% of sperms after cooling for all bulls , also individual motility% of sperms after dilution and post-thawing for bulls 1810 and 1711 in addition to no found any differences between bulls 1810 and 1827 in volume of semen and individual motility% of sperms for post-thawing . The complete results presented in Table 1, indicate that the highest properties of sperm and production number of straws were demonstrated in sire 1711 during January, February and November and bull 1810 during March and April.

TABLE 1. Individual variation in semen properties and number of straws of Holstein bulls for age 3 year during January, February, March, April and November months (Mean +SE)

Months	Bulls NO.	Ejaculate Volume	Mass activity %	Dilution rate	Individual motility %			Number of straws produced per bull
					Post dilution	Post cooling	Post-thawing	
January	1810	3.70±0.257^{B}	43.33±1.885^{B}	19.37±2.734^{B}	62.50±1.305^{AB}	58.75±1.642^{A}	54.16±1.353^{A}	304.41± 46.249^{B}
	1827	7.14±0.508^{A}	42.85±1.844^{B}	15.57±0.895^{B}	59.28±0.714^{B}	54.28±0.714^{B}	50.01±0.110^{B}	455.71±55.632^{C}
	1711	7.77±0.464^{A}	48.88±1.112^{A}	23.33±1.247^{A}	64.44±0.556^{A}	60.55±1.001^{A}	55.55±1.001^{A}	796.88±63.665^{A}
February	1810	4.20±0.800^{B}	40.01± 3.162^{B}	18.20± 2.310^{B}	57.06± 4.358^{B}	51.04±5.338^{B}	51.25±1.250^{A}	313.75± 108.406^{B}
	1827	5.57±0.719^{B}	37.14±1.844^{B}	9.14±1.078^{C}	55.01±2.672^{B}	49.28±3.350^{B}	50.01±2.002^{B}	220.66±49.634^{B}
	1711	10.23±0.211^{A}	50.54±4.679^{A}	25.43±1.875^{A}	65.54±6.432^{A}	60.12±4.621^{A}	55.22±3.205^{A}	722.54±54.761^{A}
March	1810	4.45±.376^{A}	41.66±1.667^{B}	16.33±2.178^{A}	60.83±1.035^{A}	56.25±0.897^{A}	52.50±1.151^{A}	349.36± 55.943^{A}
	1827	5.41±0.556^{A}	33.34±4.278^{C}	10.90±1.801^{B}	47.27±6.192^{B}	47.77±3.073^{B}	39.45±2.994^{B}	352.25±85.721^{A}
	1711	6.22±0.564^{A}	50.33± 5.432^{A}	15.67±1.765^{A}	55.64±6.121^{A}	50.54±2.431^{C}	50.32±3.221^{A}	232.54±44.430^{B}
April	1810	4.27±0.472^{B}	45.55± 3.379^{A}	18.88± 2.502^{A}	65.03± 1.863^{A}	60.55±2.272^{A}	56.11±2.010^{A}	434.01±78.226^{A}
	1827	7.57±0.685^{A}	37.14±2.857^{B}	8.57±1.087^{B}	62.94±1.010^{A}	57.14±1.016^{A}	51.42±0.922^{B}	349.17±64.634^{B}
	1711	5.03±0.894^{B}	42.04±3.830^{AB}	19.01±1.471^{A}	57.06±3.516^{B}	50.34±2.034^{B}	48.59±1.98^{B}	98.76±34.669^{C}
November	1810	4.28±0.406^{B}	44.28± 4.285^{A}	22.60±3.091^{A}	55.02± 2.879^{B}	60.02±2.041^{A}	53.75±1.250^{AB}	495.50±71.701^{B}
	1827	5.40±0.748^{B}	40.11±4.472^{A}	9.41±2.181^{C}	61.12±1.001^{A}	56.11±1.022^{A}	51.13±1.020^{B}	290.25±63.621^{C}
	1711	7.41±0.952^{A}	40.03±6.831^{A}	14.60±4.467^{B}	53.33±2.490^{B}	60.04±2.041^{A}	55.05±2.041^{A}	762.66±53.619^{A}

Means with different superscripts within each column differ significantly (P≤0.05).

2- Individual variation in semen properties and number of straws for age 5 year of Holstein bulls:

Effect of individual bulls on sperm motility and number of straws produced by bulls for age 5 year during January, February, March, April and November is presented in (Tables 2 , 3).

1-Volume of semen: Tables 2 and 3 show that volume of semen for bull 1818 highest significant

(P<0.05) than bulls 1819 during January (7.62,4.10), bulls1819 , 689, and bull499 during March (3.50,5.02,5.01) and also bull1819 during April (4.75) and bulls 689 and 1819 in November(5.50,5.08), moreover during April bulls 689 and 499 record more significant (P<0.05) in volume semen of bull 1819,but the others comparative no significant such as between bull 689 and bull 1819 during January, March, and November also bull 499 no differ significant from bulls 689 ,1819 and 689 during January and March and no found any differences significant between all bulls in February.

2-Mass Motility%: Results of present study revealed that mass motility% of sperm for bulls 689 and 1818 better significant (P<0.05) than bull 1819 in February (43.33,40.02,38.01) , March (42.04,41.66,19.28), April(46.66,42.24,24.87) and November(39.03,36.37,28.33) and also bull 1818 show high motility than bull 499 in January (33.75,26.02) and February (40.02,26.66),in addition to bull 1819 give mass motility% high significant (P<0.05) compare with bull 499 in January (38.00,26.02)and February (38.01,26.66) but in March (19.28,38.75) and April (24.87,47.24)opposite of that but the differences no significant such as between bulls 689 and 1818 in February , March, April and November and among bulls 1818 with 1818 in January and with 499 in March and April.

3-Dilution rate: The dilution rate has been significantly (P<0.05) increased, according to the previous study, for bull 689 compare with 1818 and 1819 in January (20.67,13.00,11.88), February (23.67,16.60,17.80) , March (19.60,13.05,8.67) and November (18.01,12.60,11.66),whereas dilution rate of semen for bull 1819 (29.80)high significantly (P<0.05) than 689 (16.16),1818 (12.56) and 499 (13.16) in April ,moreover the differ significant (P<0.05) between bull 689 and 499 in February and between 689 with 1818 and 499 in April ,but the different no significant between bulls 1818 and 1819 in months January, March and November, also among 1818 , 1819 and 499 in February and between 1818 and 499 in April.

3-Individual motility %: Individual motility % of sperm after dilution for bull 689 increased significant (P<0.05) compare with bull 1818 in January (57.51,49.37), bull 1819 in November(54.50,43,33) and both bulls 499 and 1819 in January (57.51,32.03,50.01), February(53.33,36.66,44.01), March (59.30,55.12,28.57) and April (66.61,46.46,46.43), in addition to the variance significant(P<0.05) among bull 1818 with 1819 and 499 in March. Results define that the differences no significant between bulls 1818 and 689 in February, November and March and with 1819 in January, with 499 in March and between bulls 1819 with 499 in April.

TABLE 2. Study individual variation in semen properties and number of straws of Holstein bulls born in Iraq for Artificial Insemination Center during age 5 year in months January, February and March (Mean +SE)

Months	Bulls NO.	Ejaculate Volume	Mass activity %	Dilution rate	Individual motility %			Number of straws per bull
					post dilution	post cooling	post-thawing	
January	689	5.50± 0.341^{AB}	41.67± 3.073^{A}	20.67± 1.358^{A}	57.51± 3.593^{A}	51.62± 4.409^{A}	50.01±0.110^{A}	451.22± 72.457^{A}
	1818	7.62±0.748^{A}	33.75± 2.630^{B}	13.00±3.070^{B}	49.37±3.671^{B}	47.50±5.590^{A}	51.25±1.250^{A}	409.75± 78.007^{A}
	1819	4.10±0.640^{B}	38.00±2.905^{A}	11.88±1.782^{B}	50.01±3.162^{B}	47.53±2.988^{A}	50.01±0.011^{A}	241.03±50.008^{B}
	499	6.80±0.969^{AB}	26.02± 2.449^{C}	24.50± 3.500^{A}	32.03± 3.741^{C}	30.01±3.211^{B}		
February	689	5.66 ±0.334^{A}	43.33±3.334^{A}	23.67±1.334^{A}	53.33±12.018^{A}	60.05± 5.001^{A}	55.08± 5.001^{A}	626.06± 59.003^{A}
	1818	5.60±0.509^{A}	40.02±0.010^{A}	16.60±2.461^{B}	54.02±3.204^{A}	44.02±5.113^{B}	41.00±4.295^{B}	306.60±92.957^{B}
	1819	4.60±1.166^{A}	38.01±2.011^{B}	17.80±1.019^{B}	44.01±2.449^{B}	24.01±6.011^{C}		
	499	4.66±0.333^{A}	26.66± 3.321^{C}	20.02± 2.516^{B}	36.66± 3.345^{C}	20.00±2.478^{C}		
March	689	5.02± 1.140^{B}	42.04± 2.002^{A}	19.60± 2.315^{A}	59.30± 2.449^{B}	53.05± 3.391^{B}	51.25± 1.250^{AB}	506.30± 94.244^{A}
	1818	7.50±1.024^{A}	41.66±1.667^{A}	13.05±3.286^{B}	63.33±1.668^{A}	59.16±2.006^{A}	53.23±2.108^{A}	454.38±74.717^{A}
	1819	3.50±0.681^{B}	19.28±6.308^{B}	8.67±2.484^{B}	28.57±3.921^{D}	50.01±0.011^{B}	50.02±1.012^{B}	204.51±94.501^{B}
	499	5.01± 0.577^{B}	38.57± 1.428^{A}	17.11± 2.851^{A}	55.12± 2.672^{C}	52.51± 1.118^{B}	50.11±2.023^{B}	255.33± 57.491^{B}

Means with different superscripts within each column differ significantly (P≤0.05).

TABLE 3. Study individual variation in semen properties and number of straws of Holstein bulls born in Iraq for age 5 year, in months April and November (Mean +SE)

Months	Bulls NO.	EjaculateVolume	Mass activity %	Dilution rate	Individual motility %			Number of straws per bull
					Post dilution	Post cooling	Post-thawing	
April	689	5.16 ± 0.601^{A}	46.66 ± 3.334^{A}	16.16 ± 2.227^{C}	66.61 ± 2.108^{A}	61.56 ± 2.108^{A}	60.01 ± 2.581^{A}	470.02 ± 85.581^{A}
	1818	6.83 ± 0.577^{A}	42.24 ± 4.648^{A}	12.56 ± 1.573^{B}	61.21 ± 2.321^{B}	54.45 ± 3.944^{B}	49.07 ± 3.370^{B}	378.11 ± 81.963^{A}
	1819	4.75 ± 0.773^{A}	24.87 ± 4.817^{B}	29.80 ± 1.123^{A}	46.43 ± 3.130^{C}	52.01 ± 3.391^{B}	51.25 ± 1.250^{B}	252.03 ± 54.874^{B}
	499	5.71 ± 0.178^{A}	47.24 ± 4.199^{A}	13.16 ± 1.209^{B}	46.46 ± 3.369^{C}	41.78 ± 4.564^{C}	43.11 ± 2.003^{C}	208.98 ± 66.543^{B}
November	689	5.50 ± 0.521^{B}	39.03 ± 3.145^{A}	18.01 ± 2.867^{A}	54.50 ± 4.178^{A}	53.75 ± 3.750^{B}	52.85 ± 1.486^{A}	462.71 ± 84.594^{A}
	1818	8.25 ± 0.359^{A}	36.37 ± 4.667^{A}	12.60 ± 3.218^{B}	53.34 ± 3.923^{A}	60.01 ± 2.041^{A}	53.75 ± 1.250^{A}	567.06 ± 69.693^{A}
	1819	5.08 ± 0.800^{B}	28.33 ± 4.031^{B}	11.66 ± 2.848^{B}	43.33 ± 4.098^{B}	58.34 ± 1.667^{A}	51.67 ± 1.667^{A}	267.34 ± 81.92^{B}

Means with different superscripts within each column differ significantly (P≤0.05).

Individual motility % of sperm: after cooling summarized in Table 2 and 3 revealed that after cooling the individual motility% of sperms for bull 689 increased significant(P<0.05) compare with 1818 and 1819 in February(60.05,44.02,24.01) and April (61.56,49.07,52.01) , also more significant(P<0.05) bull 499 in January (51.62,30.01), February(60.05,20.00) and April (61.56,43.11) but bull 1818 give better motility significant(P<0.05) than 1819 in February(44.02,24.01) and March (53.23,50.02) ,moreover individual motility% of bull 1819 better significant(P<0.05) than 689 in November (60.01,53.75) ,499 in January (47.53,30.01) and April (51.25,43.11), but no found any different between 689,1818 and 1819 in January and among 1819 and 499 in February in addition to the different no significant between bull 689,1819 and 499 in March and between 1818 and 1819 in November month.

Individual motility % of sperm after freezing: individual motility% after freezing in liquid nitrogen for bull 689 more significant(P<0.05) than bull 1818 in February(55.08,41.00) and 1818 , 1819 and 499 in April (60.01,49.07,51.25,43.11) but bull 1818 recorded high significant(P<0.05) in post-thawing motility % than bulls 689 and 1819 in March (53.23,51.25,50.02) and 499 in March (50.11)and April (43.11) ,also 1819 give more significant(P<0.05) than 499 in April and we knowing from present study that viability sperms lost after freezing for bull 499 during January and February also this statement show for bull 1819 in February. Table 1 and 2 revealed that the different no significant whether between 689, 1819 and 499 in March, and among 1818 and 1819 in April also the differences between all bulls in January and November expect 499 in November don't produce any good semen.

4- Number of straws: The number of straws which produced by different individual Holstein bulls born in Iraq were illustrated in Tables 2 and 3 . The Bull 689 tended to have preferable significant(P<0.05) in number of straws than the Bull 1819 in January (451.22, 241.03), March (506.30, 204.51) , April (470.02,252.03) and November (462.71,267.34) and 499 in March (255.33) , April (208.98) in addition to the differences significant(P<0.05) between bulls 1818 with 1819 and 499 in March, April and the different significant(P<0.05) among 1818 and 1819 in January ,on another hand the varied no significant between bull 689 and 1818 January, March, April and November, and among 1819 with 499 in March and April. Results in Table 1 show that bull did not produced straws in months (January and February) and this applies to bull 1819 in February. Conclusion of Table 2 and 3 indicate that the greatest level of most semen parameters and number of straws were in sire 689 in months (January, February and April) , and sir 1818 in March and November and lowest characteristics bull 499.

3- Effect age on semen properties and number of straws of Holstein bulls born in Iraq:

There was an effect of bull ages (2,3,4,5 years) on ejaculate volume, mass motility, and dilution rate, individual motility after dilution, cooling and post-thawing and number of straws which produced from bulls presented in **Table 4:** Result show that the average semen volume for age 5 years (15.18) tends to increase significant (P<0.05) compared to the age of 2,3,4 years old (3.47,5.20.6.07) respectively, moreover result revealed . The highest mean significant (P<0.05) in mass motility %, dilution rate , individual motility% of sperm after dilution, cooling , post –thawing and number of straws were recorded for bulls aged 3 (37.25,17.10,52.50,57.32,54.81,506.07) and 4 years (40.48,15.02,58.47,55.25,51.11,454.18) in compare with age 2 (17.89,8.26,15.26.20,60,15.00,75.11) and 5 year (28.04,12.19,47.38,40.27,41.96,294.05) respectively and lowest mass motility %, dilution rate, individual motility% of sperm after cooling ,dilution, post –thawing and number of straws were observed for age 2 year. However, no significant differences were observed in volume of semen for bulls age 2,3 and 4 year and also the comparative no significant between age 3 and 4 year in dilution rate and individual motility% of sperm after cooling, post-thawing and number of straws. Summarized that was the superior performance of bulls aged 3-4 years than all other age groups.

4- Effect month on semen properties and number of straws of Holstein bulls born in Iraq:

In this study, an average semen properties and number of straws of Holstein bulls born in Iraq are presented in Table 5. The maximum volume of semen / ml (7.65,7.70), mass motility % (44.82,47.20), dilution rate (17.95, 15.96), individual motility% of sperm after dilution(58.12, 58.92), cooling (55.47, 56.92), post –thawing (51.50, 53.60) and number of straws (462.25, 429.45) were found in March and April respectively and significantly different from those collected in January , February and November (P < 0.05) in all qualitative and quantitative parameters of ejaculates was confirmed in this study , in addition to volumes of ejaculates , number of straws were collected in November . (5.87,387.15) which was significantly(P < 0.05) different from those collected in January (3.34,148.40) respectively as well as individual motility% of sperm after dilution in November (48.22) more significantly (P < 0.05) than February (35.41) but the differences no significant among March and April in all characteristics which studies and between February compared with January and November in volume of semen and dilution rate, also different no significant between January and November in individual motility% of sperm after dilution , cooling and post –thawing .

TABLE 4. Effect of age on semen properties and number of straws for Holstein bulls born in Iraq. (Mean +SE)

Age (Year)	Ejaculate Volume	Mass activity %	Dilution rate	Individual motility %			Number of straws per bull
				Post dilution	Post cooling	Post-thawing	
2	3.47 ± 0.211 ^{B}	17.89 ± 1.167 ^{C}	8.26 ± 0.263 ^{C}	15.26 ± 1.929 ^{D}	20.60 ± 32.141^{C}	15.00 ± 0.023^{C}	75.11 ± 0.056 ^{C}
3	5.20 ± 0.235 ^{B}	37.25 ± 1.506 ^{A}	17.10 ± 0.912 ^{A}	52.50 ± 2.028 ^{B}	57.32 ± 1.187^{A}	54.81 ± 0.747^{A}	506.07 ± 35.359 ^{A}
4	6.07 ± 0.204 ^{B}	40.48 ± 1.037^{A}	15.02 ± 0.847^{A}	58.47 ± 1.159^{A}	55.25 ± 1.016^{A}	51.11 ± 1.035 ^{A}	454.18 ± 21.936^{A}
5	15.18 ± 1.356 ^{A}	28.04 ± 1.531^{B}	12.19 ± 0.847^{B}	47.38 ± 1.453^{C}	40.27 ± 2.052^{B}	41.96 ± 1.269 ^{B}	294.05 ± 24.573^{B}

Within Column different large letters for each parameter differed significantly (p<0.05) .

TABLE 5. Effect month on semen properties and number of straws of Holstein bulls born in Iraq (Mean ±SE).

Months	Ejaculate volume	Mass activity %	Dilution rate	Individual motility %			Number of straws per bull
				post dilution	post cooling)	post-thawing	
January	3.34 ± 0.374 ^{C}	38.12 ± 1.304 ^{B}	10.36 ± 1.234 ^{B}	43.3871 ± 1.860 ^{B}	40.37 ± 1.847 ^{B}	38.45 ± 0.313 ^{B}	148.40 ± 44.775 ^{C}
February	4.14 ± 0.397 ^{BC}	38.92 ± 1.455 ^{B}	11.82 ± 1.105 ^{B}	35.41 ± 3.028 ^{C}			
March	7.65 ± 0.470 ^{A}	44.82 ± 2.603 ^{A}	17.95 ± 1.380 ^{A}	58.12 ± 3.652 ^{A}	55.47 ± 1.190 ^{A}	51.50 ± 0.734 ^{A}	462.25 ± 39.603 ^{A}
April	7.70 ± 0.336 ^{A}	47.20 ± 2.852 ^{A}	15.96 ± 2.301 ^{A}	58.92 ± 2.856 ^{A}	56.92 ± 2.663 ^{A}	53.60 ± 2.557 ^{A}	429.45 ± 41.161 ^{A}
November	5.87 ± 0.354 ^{B}	40.25 ± 2.761 ^{B}	12.26 ± 1.619 ^{B}	48.22 ± 3.625 ^{B}	40.25 ± 2.098 ^{B}	40.63 ± 0.701 ^{B}	387.15 ± 48.653 ^{B}

Discussion

The effect of the individual bulls on the quality traits evaluated after collection (volume semen in ml, mass motility%) and steps of freezing (dilution rate, individual motility% of sperms after, dilution, and post-thawing) and calculated number of straws of Holstein bull during January, February, March, April and November months for ages 3 and 5 years are presented in (Tables 1,2,3,4). Results revealed that qualitative and quantitative parameters of Holstein bull born in Iraq semen variation between individuals for same age and this variation differences according to the variation between ages 3 and 5 years of the bulls. Internal factors are described too which effect on semen quality and quantity specially the genetic [15, 16]. Sperm quality is influenced by many factors, such as breed, individual variation and sire age [17]. The rising pace of AI in the last few decades resulted in only a few top sires being used on a wide scale [18]. The best animals grow in pedigrees and so it is nearly impossible to find animals in a certain dairy breed without many genetic ties [19]. Amann and Katz [20] Sperm tolerance to frost greatly varies between sires and also between single sires' ejaculates.

Doležalová et al. [21] reported that the results confirm the above results and validate the need for each specific sire for the individual processing technology proposed. Results of present study show individual variation in semen properties of Holstein bulls born in Iraq bulls in spite of same age and month produced semen . Significant inter-sire differences in observed spermatozoa characteristics were confirmed [21]. Also result study revealed that freezability of semen differ from bull to another' agreement with results reported by Beran et al. [17] indicated individual differences in resistance of sperm to freezing. Our findings are in agreement with Strapák et al. [22]. Ježková et al. [23] described significant effect of individuality in reproduction of sires as well as dairy cows. Beran et al. [17] verified observations of individuality and initial ejaculate content have thawed on the final consistency level of AI doses. The findings indicate major variations in the sperm volume, density and activity, as well as the amount of living and dead sperm after the collection; sperm mobility decreases in fresh ejaculate, after thawing, and during the entire thermodynamic test; and the proportion of living and dead sperm after thawing [17]. In addition, the consistency of post-thaw semen varies considerably following cryopreservation between various Bulls and even between ejaculates from the same Bull's exits [24]. The disparity in volume of semen, sperm concentration, sperm motility or pH, respectively, led to the variation in individual bulls [25]. Results in table 1,2,3,4 explain that that there are individual differences in the characteristics of the semen properties for Holstein bulls from one month to another. Mathevon et al. [26] demonstrated that the age and season interaction may significantly influence Friesian bulls' semen characteristics. The effect of seasonal factors, especially temperature, on sperm production in most bulls is significant, but the individual response to thermal stress is different.

Effect age on semen properties and number of straws of Holstein bulls:

Semen quality, as assessed such as volume of semen, mass motility, dilution rate, individual motility after dilution, cooling and post- thawing and number of straws were lowest significant for bulls collected at two year of age compared to all other age , this may attributed to the after pubertal period is generally characterized by rapid increases in both body and testicular weight [27]. The increase in semen properties at age can therefore be correlated with the increase in operation of the hypothalamico-pituitary axis and the development

of the testis simultaneously [28], in addition to Isnaini et al. [3] concluded that the age impact on the production of frozen semen in bull sires is varied between the same breeds, the production of frozen semen is increased from 2 to 5 years and is subsequently decreased by 5 years. So present study shows that best semen properties and number of straws which produced by bulls with ages 3and 4 years. Dairy bulls at 12 months of age reach puberty [29] and attain maturity at the age of 3 to 4 years [30]. These studies showed that bulls registered higher sperm defects before maturity. It is presumably because the testes are still growing in younger bulls and thus the sperm in the ejaculate are of poor quality [29]. The degenerative changes in seminiferous tubules may be associated with the reduced semen output in older bulls [31], deposition of fat that may occur in scrotum [32] and Body tissue breakdown, especially testicular tissues [33] with advancement in age. Fat deposition will take place around the scrotum as a bull progresses in age. This can influence the quality of semen by reducing scrotal neck heat radiation [8].

Effect month on semen properties and number of straws of Holstein bulls:

The results of the current study, which relate to showing the effect of some months of the year, according to what is available from information from the Artificial Insemination center, where the study included the effect of January and February of the winter season, March and April of spring semester, and November of Autumn season, as shown in a Table 5. It is clear from this that the months effect on semen properties and number of straws which produced from Holstein bull born in Iraq, some authors found that some semen characteristics were influenced by this monthly variability, and some had none, so there are no successive modern studies in this field, as these ancient years have an impact on bull reproductive performance [34]. Besides the study, it has been concluded that there are substantial variations in traits over months, that changes in external environmental factors such as photoperiod can be reported as they change over months [35]. The most important factors influencing semen production are external conditions, including seasonal effectiveness, stable microclimate and sampling techniques. As mating takes place during the hottest months, bulls are subject to variations in the climate that conflict with their fertility and reproductive efficacy [36]. The study of Jimenez-Severiano et al. [37] also revealed In Spring and in Winter it was recorded the highest LH values of young bulls, and in the spring and summer the highest average LH levels were reported, as were the lowest LH levels of cold seasons. The same result was seen in bulls of beef breeds from the tropics in which the consistency of semen has been decreased and testicular dimensions have been decreased in winter [38]. It is believed that not only the temperature reported on the day of semen collection, but also the temperature recorded during the whole spermatogenesis cycle up to 70 days before collection, affects the production. Therefore, superior morphological properties were found in winter and spring compared to summer and autumn for young bulls. Besides their study results, which showed that the characteristics of the semen were better in the temperature of moderately hot and cold and stronger in cold months than hot, in January and February were considered the coldest in Iraq so the properties of the semen were lower than those of March and April during these months. Muiño et al. [39] had emphasized that The consistency in semen in the cold months was higher than in hot months, but the effect on spermatogenesis could be very cold because of cold stress, photoperiod and the feed consistency may also have a negative effect. On the other hand, it was observed that the results were less in a November, perhaps because this month is the beginning of the production frozen semen.

Conclusion

- The complete results presented in indicate that the highest properties of sperm and production number of straws for bulls with age 3 years were demonstrated in sire 1711 during January, February and November and bull 1810 during March and April, but bulls with age 5 years for 689 in months (January, February and April) , and 1818 in March and November and lowest characteristics bull 499. It means that there are individual differences in the characteristics of the semen properties and number of straws for Holstein bulls from one month to another

- Present study show that bulls with age 3-4 year produced best semen properties and number of straws and the highest characteristics during months March and April.

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Conflict of interest

There are no conflicts of interest to be declared.

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التباين الفردي في صفات السائل المنوي وعدد القصبات الذي تنتجها ثيران الهولشتاين المولودة في العراق خلال أعمار وأشهر مختلفة

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الخلاصة:

هدفت هذه الدراسة إلى تحديد الفروق الفردية بين ثيران الهولشتاين المولودة في العراق في السائل المنوي الطازج والمحفوظ بالتبريد. علاوة على ذلك، تمت دراسة تأثير العمر (2،3،4،5 سنوات) والأشهر (يناير، فبراير، مارس، أبريل، ونوفمبر) على جودة السائل المنوي الطازج والمحفوظ بالتبريد. أجريت الدراسة في قسم التلقيح الاصطناعي التابع لوزارة الزراعة العراقية. كانت بيانات السائل المنوي الطازج المؤهل هي حجم القذف ونسبة نشاط الكتلة، في حين كانت بيانات السائل المنوي المحفوظ بالتبريد هي معدل التخفيف، ونسبة الحركة الفردية بعد التخفيف، وبعد التبريد، وبعد الذوبان وعدد القش المنتج من ثلاثة عشر ثيران هولشتاين تتراوح أعمارهم بين 2-6 سنوات. السنوات المستخدمة لإنتاج السائل المنوي في الأشهر المذكورة أعلاه من عام 2022. وأظهرت النتائج وجود اختلافات في إنتاج السائل المنوي الطازج (حجم القذف، نسبة النشاط الكتلي) وإنتاج السائل المنوي المجمد (معدل التخفيف، نسبة الحركة الفردية بعد التخفيف والتبريد وما بعد الذوبان، وعدد من القش) كانت معنوية بين مفردات ثيران الهولشتاين المولودة في العراق لمدة 3 و 5 سنوات والتي لا يزال التحليل هنا ينطبق عليها في التلقيح الصناعي. علاوة على ذلك، أظهرت بعض الثيران بعمر 3 سنوات أفضل جودة للسائل المنوي وإنتاج السائل المنوي المجمد في أشهر يناير وفبراير ونوفمبر، والبعض الآخر في مارس وأبريل، بينما كانت الثيران بعمر خمس سنوات في يناير وفبراير وأبريل، و أخرى في مارس ونوفمبر. كما أظهرت النتائج الحالية أن الشهر والعمر لهما تأثير معنوي على خصائص السائل المنوي وعدد القش المنتج، حيث وجد أن أفضل الصفات عند عمر 3-4 سنوات مقارنة بعمر 2 و 5 سنوات وأن أفضل الصفات عند عمر 3-4 سنوات مقارنة بعمر 2 و 5 سنوات وأن شهر مارس وأبريل أفضل من أشهر يناير وفبراير ونوفمبر.

الاستنتاج: تظهر هذه الدراسة تباينا واضحا في نوعية السائل المنوي الناتج عن الفروق الفردية بين ثيران الهولشتاين المولودة في العراق، والثيران بعمر 3-4 سنوات أنتجت أفضل خصائص السائل المنوي وعدد القش. علاوة على ذلك فإن أعلى خصائصه تكون فقط في شهري مارس وأبريل.

الكلمات المفتاحية: الاختلافات الفردية، ثيران الهولشتاين، القصبات المجمده.