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Effect of Season and Age on Semen Characteristics and Physiological Changes in the Testis of Jonobi Bull Breed in Iraq.

Falah A. AL-Asadi, Waleed.Y. Kassim and Amad F. Hassan

Department of Animal Production, Agricultural College, University of Basrah, Iraq.

Abstract The study was conducted in the physiological laboratory - Department of Animal Production of the Agriculture college- Basra University, from 11/2/2017 to 2/1/2018, to investigate the relationship between two factors (age and seasons) and the characteristics of semen (pH, sperms concentration, mass and individual motility, live and dead sperm, testosterone level, AST, ALT and ALP enzymes concentration) of Jonobi bull breed in southern of Iraq. A total of 12 samples of Jonobi bulls were used, aged 2-4 years old. The results showed that there was a significant ($P<0.05$) increase in the pH values, AST, ALT and ALP enzymes concentration in the semen of younger bulls (2 years old) in comparison with the older ones (4 years old). Whereas, the mass and individual motility, live sperm, sperms concentration and testosterone level increased significantly ($P<0.05$) in the semen of older bull (3 and 4 years old) in comparison with younger animals (2 years old). The pH values, AST, ALT and ALP enzymes concentration were increased significantly ($P<0.05$) in spring season as compared with the other season, while, the mass and individual motility, live sperm, sperms concentration and testosterone level increased significantly ($P<0.05$) in the summer and autumn seasons compared to other seasons.

Key words:season, age, semen characteristics, bulls.

Introduction

The quality and quantity of sperm depend mainly on many factors such as environmental conditions, feeding system, management and health of bulls (1). Genetic factors also play a significant role in the quality of sperm through genes inherited from parents to sons over several years of life (2). Furthermore, the health and age of bulls and semen collection techniques are active in influencing sperm quality (3).

Season also has an effecting role on semen, as well as bad management that may be negatively associated with semen collection technologies and unsuccessful selection of breed (4,5,6,7).One of the most important environmental factors affecting the characteristics of bulls' semen is the high temperature, especially during the summer season, which causes negative effects on the secretion of the release hormones factors (GnRH) and the reduction of the concentrations of the hormones FSH and LH as a direct result of the animal's exposure to heat stress, and this may lead to a decrease in the efficiency of Spermatogenesis and higher production of weak

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and abnormal sperms (8 , 9). Also, the high temperatures lead to an increase in the rates of corticosteroid secretion in the blood and to reduce the rates of thyroxine secretion from the thyroid gland In addition, the high temperatures lead to a decrease in the quality of the feed and its low content of carbohydrate, protein and vitamins, and it reduces the feed consumption, which leads to failure to meet the animal's nutritional requirements and impacts on the quality of the bull's semen (6,10,11)

Due to a few of available reports about the effect of age and season on the semen of Jonobi bulls breed in Iraq, this study aimed to find out the best seasons and ages that can give the highest quality of bull`s semen.

Materials and methods

This study was conducted in the physiological laboratory - Department of animal production of the agricultural collage - University of Bassora, from 11/2/2017 to 2/1/2018. A total of 12 samples of Jonobi bulls breed were used in the study, ranging from 2-4 years old after their slaughter in the animal`s massacre of Al-Zubair town - Basrah province . The testes were separated from the carcass and washed with clean water and placed directly into nylon sacks containing distilled water and transported to the laboratory. After the samples arrived at the laboratory, the scrotum was separated from the testes and 2 ml of saline solution at a concentration of 2% with a temperature 25 ° C was injected into the tail area of the epididymis, then, the sperm was withdrawn and placed in Petri dishes by using a sterile medical syringe and incubated at a temperature 30 ° C. A drop of sperm was placed on a clean, warm slide under the microscope and examined at a magnification of 100. The mass motility, individual motility, percentage of live and dead sperms were determined according to (12). The sperm concentration was recorded using the haemocytometer chamber (13).

The semen plasma was collected by centrifugation for 15 minutes at 4000 rpm, then the top layer (plasma liquid) was pipetted out while leaving the precipitate. Samples were frozen at -18 ° C until analysis.

The concentration of Aspartate transaminase (AST) and Alanine aminotransferase (ALT) enzymes were measured by using a chemical kit of the United Kingdom Randox Company. The concentration of Alkaline phosphatase enzyme (ALP) was determined by using a chemical kit of France BIOLABO SACompany. Testosterone was determined using a procedure combined with the kit from the Bio Merieuxsa Company - France.

The statistical program (14) was used to analyze the data statistically. The significance of the differences between the averages of the examined traits was tested using the least significant difference (R.L.S.D) at a significant level of $P < 0.05$.

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Results and discussion

Table (1) indicates a significant effect (P <0.05) for age on the pH values of bull semen, since, the bulls at 2-years old were significantly(P <0.05) higher in the pH values as compared with the bulls at 3 and 4 years old, the averages were 7.33, 7.11, 6.95, respectively. This differences in the pH values of bull semen may be due to the variation in sperm movement from age to another, whenever, more sperm movement increases, the more energy it consumes (fructose) and the pH becomes acidic, whereas. the less sperm movement, the less energy consumption and the pH is close to the basic point, and this supports the findings of our data in Table (1), which, the mass and individual motility of the bull`s sperm at the age of 4 years was higher than the values of bulls at of 2 years old and reached (74.54, 75.80)% and (65.42, 66.86)%, respectively, which made the pH values of the first age less than the second. While, the reason of the significant increase in the mass and individual motility of the sperms in the older animals than younger once (Table 1), it may be due to increasing the secretion of testosterone from the leydig-cells associated with the animal ages rising, and this hormone has an active role in increasing the size and development of testes in males and improving all sperm characteristics as it stimulates the sperm to consumption more energy needed for movement (2,11).It is known that animals at older ages consume more feed as a result of the increase in the size of the digestive system and the rumen, and this means that their bodies are supplied with more energy, which is reflected positively on the increase in sexual activity (8,9)

The pH values of Jonobi bull semen during all ages were significantly higher in spring (P <0.05) compared to other seasons, while the sperm concentration and mass and individual motility of sperm increased in the summer and autumn seasons compared to other seasons. The reason for this difference may be due to the variation in the temperature during the different seasons of the year and this was confirmed with (15,16,17).

Table 1: Effect of season and age on semen pH, sperm concentration, mass and individual motility of Jonobi bulls.

Age	Season	trails			
		pH	Sperms concentration (10^9 x ml)	Mass motility (%)	Individual motility (%)
2 years	Spring	7.81^{a}±0.23	2.33^{c}±0.10	54.38^{d}±1.97	55.51^{d}±2.13
	summer	7.05^{c}±0.14	3.76 ±0.10^{a}	72.67^{a}±2.31	74.79^{a}±2.12
	Autumn	6.90^{d}±0.17	2.80^{b}±0.17	70.74^{b}±0.72	71.68^{b}±2.17
	Winter	7.35^{b}±0.28	0.40±2.14^{d}	63.90^{c}±1.98	65.48^{c}±2.20
Mean		**7.33^{A}±0.31**	**2.47^{B} ±0.33**	**65.42^{C}±2.14**	**66.86^{C}±2.24**
3 years	Spring	7.52^{a}±0.18	2.64^{c}±0.08	59.55^{d}±1.82	60.61^{d}±2.15
	summer	6.84^{c}±0.35	2.75^{b}±0.13	80.08^{a}±2.64	81.55^{a}±2.10
	Autumn	6.72^{c}±0.31	3.08^{a}±0.23	74.20^{b}±1.31	75.62^{b}±1.94

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	Winter	7.20^{b}±0.18	2.21^{d}±0.24	68.88^{c}±2.11	69.88^{c}±2.13
Mean		**7.11^{B}±0.32**	**2.64^{A}±0.36**	**70.68^{B}±2.11**	**71.92^{B}±2.15**
4 years	Spring	7.42^{a}± 0.16	2.17^{c}±0.14	60.80^{d}± 2.14	62.28^{d}± 2.17
	summer	6.83^{c}±0.20	2.53^{b} ±0.33	84.50^{a}± 1.93	85.62^{a}± 2.05
	Autumn	6.62^{c}±0.21	2.66^{a}± 0.13	79.09^{b}± 1.51	80.06^{b}± 1.96
	Winter	7.11^{b}±0.18	2.00^{d}± 0.23	73.78^{d}± 2.10	75.28^{c}± 2.11
Mean		**6.95^{C}± 0.27**	**2.40^{B}±0.37**	**74.54^{A} ±2.23**	**75.80^{A}±2.11**

Different small and capital letters within a column and class means significant difference (P<0.05) between seasons and ages groups respectively.

Table (2) shows a significant (P <0.05) increase in the percentage of live sperm and a significant (P <0.05) decrease in the dead sperm percentage in the semen of bulls at 4 years old in comparison with 2 and 3 years old of bulls. Mean values were 82.76, 79.07 and 75.57%, respectively. Also, the dead sperm percentage decreased to 8.46% and 9.51% as compared to 10.56%, respectively, may due to the large size of the testes and the elevate in the rates of secretion of the hormone testosterone with the increase in animal`s age, additionally, the increase in the rates of feed intake by older animals than the younger, all these conditions lead to an improvement in the quality of the semen and an increase in the percentage of live sperms (1). It is also noted that testosterone concentration was significantly (P <0.05) higher in the serum of 4 years old bulls compared to 2 and 3 years old bulls (2.00, 1.91 and 1.78) ng / ml respectively (Table 2). The reason for this difference may be due to that the Leydig-cells in older bulls have more activity in the production of the hormone testosterone by converting cholesterol into steroid hormones in the testicular tissue of the animal (3).

Table (2) noticed that the season has a significant (P <0.05) effect on the percentage of live and dead sperms, as it appears that autumn and summer seasons recorded the highest means in the percentage of live sperms compared to the other seasons of the year at all ages, also, the dead sperms during the autumn season recorded lower values compared to the other seasons. The reason for this is due to the moderate temperatures during the autumn season, especially in the southern regions of the country, which positively affect the improvement of the animals' active sexual and increase in the rates of secretion of sex hormones, lead to an improvement of all the characteristics of the semen. This is confirmed by many researchers such as (8,15,17,2) in their studies about the effect of season on the quality of bull semen. While, the concentration of testosterone hormone in the serum was higher in the autumn compared to other seasons. These results are in agreement with the findings of (17,2) they reported that the concentration of testosterone varies according to the difference in the temperatures during year`s seasons.

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Table 2: Effect of season and age on live and dead sperm in semen and the concentration of testosterone in serum of the Jonobi bull breed.

Age	Season	trails		
		Live sperm (%)	Dead sperm (%)	Testosterone concentration (ng/ ml)
2 years	Spring	$68.00^{d}\pm2.17$	$13.37^{a}\pm1.50$	$1.31^{c}\pm0.04$
	summer	$78.05^{b}\pm 2.83$	$10.55^{b}\pm 1.73$	$1.95^{a}\pm0.27$
	Autumn	$81.99^{a}\pm2.18$	$8.10^{c}\pm0.83$	$2.23^{a}\pm0.55$
	Winter	$74.24^{c}\pm2.28$	$11.17^{b}\pm 0.96$	$1.63^{b}\pm 0.34$
Mean		**$75.57^{C}\pm6.28$**	**$10.56^{A}\pm1.87$**	**$1.78^{B}\pm0.49$**
3 years	Spring	$73.69^{d}\pm1.95$	$11.62^{a}\pm0.91$	$1.37^{c}\pm0.07$
	summer	$81.86^{b}\pm 1.55$	$9.18^{c}\pm 0.96$	$2.13^{a}\pm0.31$
	Autumn	$83.64^{a}\pm 2.01$	$7.75^{d}\pm1.11$	$2.40^{a}\pm0.56$
	Winter	2.89 ± 77.20^{c}	$10.27^{b}\pm1.27$	$1.72^{b}\pm0.39$
Mean		**$79.09^{B}\pm4.34$**	**$9.51^{B}\pm1.59$**	**$1.91^{A}\pm0.54$**
4 years	Spring	$77.36^{d}\pm2.36$	$10.88^{a}\pm 0.82$	$1.46^{c}\pm0.08$
	summer	$84.93^{b}\pm2.69$	$7.50^{c}\pm0.95$	$2.26^{a}\pm0.37$
	Autumn	$87.77^{a}\pm 2.36$	$6.70^{d}\pm 0.87$	$2.51^{a}\pm0.60$
	Winter	$80.97^{c}\pm2.82$	$9.42^{b}\pm0.98$	$1.78^{b}\pm0.40$
Mean		**$82.76^{A}\pm5.00$**	**$8.46^{C}\pm1.82$**	**$2.00^{A}\pm0.57$**

Different small and capital letters within a column and class means significant difference ($P<0.05$) between seasons and ages groups respectively.

Table (3) shows that age has a significant ($P <0.05$) effect on enzymes concentration (AST, ALT and ALP) in seminal plasma. All enzymes were significantly higher ($P <0.05$) in bulls 2 years of age than in bulls 3 and 4 years of age. The means of AST reached to 42.06,48.12,56.13 IU / L, ALT 30.12,36.18,40.13 IU / L and ALP 107.03,131.67,163.899 IU / L respectively. The reason for this difference in enzymes concentration is directly related to the percentage of live sperms (negative relationship) and dead sperms (positive relationship), and because age has a direct effect in determining the percentage of live and dead sperms (18,19,1,3)our data study showed the percentage of live sperms increased in the older bulls compared to the younger ones, so naturally, enzymes concentration will change according to the age of the animals.

There is a significant effect ($P <0.05$) of seasons on enzyme levels (AST, ALT and ALP). The spring and winter seasons recorded the highest values in the concentration of enzymes as compared with other seasons during the year, may be due to the variation in temperatures during the seasons of the year and their impact on the physiological state of the animal, especially the concentration of sex hormones, and

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this will lead to changes in the components and characteristics of the semen including the studied enzymes, this result has agreed with the results of (2,20,21)

Table 3: Effect of season and age on the concentration of AST, ALT and ALP (UI/L) enzymes in the seminal plasma of the Jonobi bull breed.

Age	Season	trails		
		AST	ALT	ALP
2 years	Spring	64.23^{a}±7.11	44.05^{a}±2.74	218.55 ^{a}±10.71
	summer	46.66^{c}±5.98	38.37^{c}±2.15	138.09 ^{d}±15.30
	Autumn	46.10^{c}±7.15	37.09^{c}±3.04	142.09^{c}±9.72
	Winter	59.36^{b}±7.02	40.10^{b}±3.01	163.46^{b}±8.73
Mean		**56.13^{A}±7.13**	**40.13^{A}±3.22**	**163.89^{A} ±12.17**
3 years	Spring	67.18^{a}±6.20	41.02^{a}±2.11	164.29^{a}±8.44
	summer	42.47^{c}±5.62	32.82^{c}±3.02	118.25^{d}±12.40
	Autumn	41.08^{c}± 6.31	30.24^{d}±2.77	123.07^{c}±6.50
	Winter	50.83^{b}±6.18	35.48^{b}±2.57	138.42^{b}±6.09
Mean		**48.12^{B}±6.18**	**36.18^{B} ±2.24**	**131.67^{B} ±7.15**
4 years	Spring	49.34^{a}±6.34	34.75^{a} ±2.68	137.34^{a}±6.48
	summer	35.79^{c}±4.21	25.13^{c}±2.42	98.49^{d}±10.65
	Autumn	35.53^{c}±5.11	25.08^{c}±2.34	115.13^{c}±6.67
	Winter	47.09^{b}±6.14	30.25^{b}± 2.76	103.39^{b}±8.67
Mean		**42.06^{C}±5.03**	**30.12^{C}±2.06**	**107.03^{C}±6.12**

Different small and capital letters within a column and class means significant difference (P<0.05) between seasons and ages groups respectively.

Conclusions

From the data of this study, it can be concluded that the semen characteristics of Jonobi bulls breed in Iraq are affected according to age and season, since, the high pH and most of enzymes of the seminal plasma are associated with young ages, while the other traits such as the mass and individual motility, live sperm, sperms concentration and testosterone level increase significantly with the large ages of bulls. Also, some traits improve in the spring season (pH, AST, ALT and ALP enzymes), while, others improve in the summer and autumn seasons such as the mass and individual motility, live sperm, sperms concentration and testosterone concentration. Therefore, to obtain good quality semen for artificial insemination from Jonobi bulls breed it is preferable to collect it during the summer and autumn seasons and at older ages (more than 3 years).

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