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Effect of Age and Month on Semen Characteristics and Some Biochemical Parameters and Hormones of Iraqi Buffalo Bull

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Abstract: This study was conducted in the Physiology Laboratory - Department of Animal Production of the College of Agriculture - University of Basra, Karma Ali site. Physiology Laboratory - College of Veterinary Medicine -University of Basrafor the period of1/12/2022And up to1/4/2023As used in the study buffalo bulls by agesIt ranged from 3-5 years after being slaughtered by the massacre, I separated the two testicles from carcass. They were washed with clean water and placed directly in nylon bags containing distilled water and brought to the laboratory,All semen characteristics were estimated using a semen analysis deviceSemen analysis)), which includes individual movement of all types, as it included tortuous sperm movement (VCL), straight sperm movement (VSL), sperm track movement rate (VAP), progressive linear movement (LIN), and twitching sperm movement (WOB). The sperms were also calculated in percentages of live, dead, and deformed sperms. Also, some biochemical components (total protein, albumin and globulin) and some hormones (testosterone and FSH) ,the results of the study were that the bulls at the age of 5 years were significantly superior(0.05(P<on bulls at the age of 3 years in all semen characteristics (individual movement of sperm, concentration of sperm, and types of movement).VSL ,VAP, LIN The percentage of live sperms) also decreased significantly(0.05(P<Percentage of dead and deformed sperm and twitching sperm movement(WOB)In bulls at the age of 5 years compared to bulls at the age of 3 years, and in contrast, the levels of total protein, albumin and globulin, as well as the hormones testosterone andFSHMorale rise(0.05(P<in the serum of 5-year-old bulls compared to 3-year-old bulls. The months of December and January were also significantly superior (0.05(P<For the months of February and March, in all semen characteristics, chemical components, and hormones, and at both ages, for bulls.

Keywords: buffalo bulls, age, month, semen characteristics, biochemical, hormones.

1. Introduction:

The quality of semen depends mainly on the moderation of macro and microclimatic conditions, regular feeding regime, general administration and health care of bulls, as well as the reproductive performance of bulls depends on the possibility of resistance of these animals to all these conditions, including genetic factors, which have the largest role in determining the characteristics of semen of bulls, as genetic influences play an important role in influencing the quality of semen through genes inherited from the bull's fathers and for several years of the life of the bull son (Koivisto *et al.*, 2009). The health and age of bulls and the skills and techniques of semen collection has an active role in influencing the quality of semen as well as the aspect of high temperatures (heat stress), especially in the tropics at the stage of sexual maturity of bulls may lead to a deterioration in the efficiency and work of the testicles and their production of deformed sperm and thus decrease the reproductive ability of bulls (Bhakat *et al*. ,2009 and Ahmad *et al.* ,2011 and Rehman *et al*. ,2012).

Since buffalo animals are seasonal animals in their reproduction, so many studies indicate a decrease in the reproductive efficiency of buffalo bulls with high temperatures, especially during the summer months, and the length of the photoperiod has a significant impact on the reproduction of animals through the secretion of

the hormone melatonin and the effect on the pituitary gland in the secretion of hormones feeding the gonads (Ibrahim *et al*. ,2012and AL-Sahaf *et al*.,2012).

Hence the importance of controlling most environmental conditions, the most important of which are high temperatures, providing appropriate feed for the health of bulls, and providing protein-rich feed, especially during the breeding season, to achieve good semen production for these animals and raise their reproductive efficiency (Mandal *et al*., 2000, Bhoite *et al*., 2005, Waltl *et al*., 2006, Chanhan, 2007, Koonjaenak *et al*.,2007 and Fiaz *et al*., 2010).

Also, the available reports on the effect of the bull, breed, age and season on the quality and quality of semen of buffalo bulls in Iraq are few and do not raise the required level for this animal, which is one of the most sacred animals in Iraq, so the aim of this study was to identify and know the best seasons and ages that can give the highest quality and quality of the semen of buffalo bulls in Iraq.

2. Materials and Working Methods:

Sample Collection:

This study was conducted in the Physiology Laboratory - Department of Animal Production of the College of Agriculture - University of Basra, Karma Ali site, and the Physiology Laboratory - College of Veterinary Medicine - University of Basra for the period from 1/12/2022 to 1/4/2023, as buffalo bulls were used in the study with ages ranging from 3-5 years after slaughter in the massacre (Basra massacre on the road linking the Zubair area with Basra Governorate) The two characteristics were separated from The carcass was washed with clean water and placed directly nylon bags containing distilled water and brought to the laboratory, after the samples arrived at the laboratory, the scrotum was separated from the testicle and 2 ml of saline solution at a concentration of 2% andat a temperature of 25 ° CO in the tail area of the epididymis.

Measuring semen traits:

Then I pulled the sperm using a sterile medical syringe and placed in Petri dishes and incubated at a temperature of 30 °C O in the incubator, and took a drop of semen on a clean and warm glass slide at a temperature of 37 ° C and covered with the lid of the slide and placed under the lens of the microscope with a magnification power of 100x and estimated all the characteristics of semen using a semen analysis device which individual movement of all kinds as it included the movement of winding sperm (VCL and straight sperm movement (VSL) Sperm pathway movement rate (VAP), progressive linear motion (LIN) and twitching movement of sperm (WOB) as well as sperm were calculated by percentages of live, dead and deformed sperm according to the method (Chemineau et al.,1991) and using the pigments of eosin and crocin, where a drop of semen is placed at the tip of the glass slide from the top and a drop of eocene dye and a drop of dye are placed over it Nicrosin and mix well with the drop of semen and make a swab with another slide and leave to dry for 5 minutes, then examined under the microscope with a magnification power of 400X, search I took dead sperm violet color while the live sperm remained in its transparent color, and calculated 200 sperm in different fields of the slide in the shape of the letters, and in the same way the deformed sperm was calculated and the sperm deformities receded in three axes, deformities of the head, tail and middle piece.

Collection of blood samples and some biochemical components and hormones:

Blood samples were collected during the slaughter of animals in test tubes containing gel for the purpose of separating the serum, brought to the laboratory and placed in a centrifuge at a speed of 3000 cycles / minute for 15 minutes, after which the formed serum was withdrawn using a sterile medical syringe, and the serum was placed in clean and sterile test tubes for the purpose of conducting all analyzes of biochemical components and hormones.

The total protein was measured using the diagnostic kit produced by the United Kingdom Randox Company, and the light intensity was measured using a spectrometer at a wavelength of 546 nm, and the total protein content was estimated using the equation:

Total protein concentration in g / 100ml =

$$\frac{Sample\ absorbanse}{Standard\ protein\ absorbency} \times \mathbf{0.6}$$

The albumin was measured using a diagnostic kit from Randox UK, the light intensity was measured using a spectrometer at a wavelength of 546 nm, and the albumin content was estimated using the equation:

Albumin concentration g / 100ml =

$$\frac{Sample\ absorbanse}{Standard\ solution\ absorbency} \times \mathbf{4.5}$$

According to coupling using the equation:

Globulin concentration/100ml = Total protein concentration – Albumin concentration

The concentration of testosterone in the blood serum of the experimental bulls was measured using the Mini visas device manufactured by the French company bio Merieuxsa, and using the kit manufactured by the same company.

Statistical analysis:

The data is analyzed statistically using the ready-made statistical program (SSPS, 2019) as a factorial experiment that includes the first factor the effect of the age of the animal (bulls aged 3.5 years), and the second factor, which includes the effect of the months of the year (December, January, February, March) in all studied traits, which included semen characteristics and some biochemical components and hormones of the Iraqi local buffalo bulls and according to the following mathematical model equation:

Yijk=M +Ti +Bj +TBij + eijk

Whereas:

Yijk = represents the studied adjective
M = Overall Mean
Ti = Bull Age Effect
Bj = Effect of month of year
TBij = Overlap between age and month
eijk = experimental error.

The significance of the differences between the averages of the studied traits was tested using the least significant difference (R.L.S.D.) and at the level of significance (P<0.05).

3. Results and discussion

The table shows that (2.1) for the age of bulls has a significant effect (P<0.05)in the characteristics of semen, as it is noted in Table (1) that bulls at the age of 5 years significantly outperformed (P<0.05)on bulls at the age of 3 years in the concentration of sperm, and the individual movement of sperm as it reached 2. 16,1.85 ×109 / ml and 59.62,52.21% respectively, as well as the movement of winding sperm (VCL) decreased significantly (P<0.05)in the semen of bulls aged 5 years compared to bulls aged 3 years, as it was 49.73, 56.34 µm/s respectively, and it is noted from Tables (1,2) height The progressive movement of sperm (VSL) and the rate of sperm path (VAP) are significant (P<0.05)for bulls aged 5 years, as it was 37.80, 32.66 µm/s,

compared to bulls aged 3 years, as it recorded 33.48, 29.84 µm/s. Also from Table (2), the sperm of 5-year-old bulls excelled significantly (P<0.05)on bulls aged 3 years in the percentage of straight progressive sperm movement (LIN) as it was 33.41 and 30.61% respectively, while the percentage of winding sperm movement (WOP< decreased significantly for bulls aged 5 years compared to bull sperm aged 3 years and was 52.26 and 59.11% respectively Also, from Table (2), it is clear that the sperm of bulls aged 5 years significantly outperformed (P<0.05)compared to bulls aged 3 years in the percentage of live sperm, which was 54.93, 47.48% respectively, and in contrast, the percentage of dead and mutilated sperm of bulls aged 5 years compared to bulls aged 3 years decreased significantly (P<0.05)percentages of dead and mutilated sperm compared to bulls aged 3 years, recording 14.99, 16.91, 11.67, and 13.21% respectively. The reason for this difference in the characteristics of semen at different ages of buffalo bulls is due to the large size of the testicles and increase their productive and secretory activity , and the size and number of cells for your hand and increase the concentration and secretion of testosterone from them with the age of the animal, and that this hormone has an active role in improving all the characteristics of Semen as it stimulates sperm to increase its processing of the energy necessary for movement by making the most of fructose sugar and proteins present in sperm plasma (Ghosh, 2004, Bhakat et al., 2009 andAhmad et al., 2011), as well as increasing the rates of feed intake by animals at older ages compared to At a lower age, increased secretion of growth hormone and thyroxine, and thus increasing its resistance to stress types, all these conditions lead to an improvement in the quality of semen, an increase in the percentage of live sperm, and a decrease in the percentage of dead and deformed sperm (Mandal et al., 2005 and Bhakat et al.,2011 and Farooq et al.,2013)

Also, from the two tables (1,2) it is noted that the month of the year has a significant effect (0.05 (P< in the characteristics of the semen of the Iraqi local buffalo bulls, as it is clear in the joule (1) that the months of January and March are a significant superiority (P<0.05) over the months of February and December in the concentration of sperm and were 2. 43,2. 53 f,2. 24, 2.09 ×109 / ml for the ages of bulls 5 and 3 years respectively, as shown from Table (1) that the months of December and January significantly superiority (P<0.05)over the months of March and February in the percentage of individual movement of sperm, as it was 67.89, 65.64 and 62.18, 54.89% for the ages of bulls 5 and 3 respectively, as well as the month of January recorded a significant decrease (P<0.05) significantly in the movement of zigzag sperm (VCL) in the semen of bulls at the age of 5 years and bulls at the age of 3 years compared to the rest of the months were 43.27, 54.22 µm/s respectively, and Table (1) shows that the months of January and December have a significant superiority (P<0.05)over the rest of the months in the progressive movement of sperm (VSL) was for bulls aged 5 years 44.74, 41. 16 µm/s for 3 years 37.19, 34.18 µm/s and vap respectively for bulls aged 5 years 34.37, 33.18 µm/s and for 3 years 31.73, 30.17 µm / s It is noted from Table (2) that the months of December and January recorded the highest significant ratios (P<0.05)in the percentages of straight progressive sperm movement (LIN). As the bulls were 5 years old 37.12, 34.22% and bulls aged 3 years 33.11, 32.85% respectively, while the month of January recorded the lowest percentages significantly (P<0.05) in the percentage of the movement of winding sperm (WOP) for bulls at the age of 5 years was 50.16% and for bulls at the age of 3 years was 58.73% compared to the rest of the months. It is also clear from Table (2) that the months of December and January have recorded the highest significant ratios (P<0.05)in the percentage of live sperm, so it was for bulls aged 5 years 61.11, 57.15% and bulls aged 3 years 52.17, 49.23 % respectively compared to the rest of the months, and in contrast from the same table, it is noted that the months of December and January recorded the lowest significant ratios (P<0.05)In the percentage of dead and deformed sperm, the ages of bulls 5 years were 13.92, 14.42 and 9.17, 11.24% and the ages of bulls 3 years were 14.82, 14.95 and 11.78, 12.96% respectively compared to the rest of the months , and the reason for this improvement in the characteristics of bulls sperm during the months of December and January may be due to moderation The climate in Iraq during the winter season is characterized by

moderate cold, moderate temperature and humidity, which reflects positively on the increase in sexual activity of the testicles (Bhat et al. , 2004 and Mulugeta et al. 2006) and (Helbig et al. 2007) On the other hand, the shortening of the photoperiod and the increase in the hours of darkness, which has the greatest impact on increasing the secretion of the hormone melatonin, which increases the effectiveness and activity of your cells to secrete testosterone, and many researchers confirmed about the effect of the season and temperature variation that it has an effective effect in determining the sexual activity of many males. of animals, including buffalo bulls, as they showed that the variation in temperature and short daylight hours have the greatest impact on determining the secretion of sex hormones, especially testosterone ,increasing the activity of testicles figs, and improving semen characteristics in bulls (Waltl et al., 2006 and Chanhan, 2007 and Koivisto et al.,2009 and Ibrahim et al .,2012).

Table (1) shows the effect of bull age and month on sperm concentration, single, zigzag and straight movement and sperm trajectory rate.

Age groups	Months	Epithets				
		Sperm concentration ($\times 10^9$)	Individual movement of sperm (%)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)
3 Years	December	0.04±1.19c	2.44±62.18a	3.02±52.16cd	1.97±34.18a	1.97±30.17a
	January	0.06±2.24a	2.51±54.89a	2.93±54.22c	2.11±37.19a	1.83±31.73a
	February	0.05±1.87b	2.85±44.23c	2.83±61.34a	2.17±30.82b	1.92±28.11b
	March	0.06±2.09a	2.65±47.17c	3.05±57.18b	2.09±31.73b	2.11±29.34b
Average		0.05±1.85B	2.57±52.21B	2.78±56.34A	2.11±33.48B	2.03±29.84B
5 Years	December	0.07±1.55c	2.34±67.89a	2.73±46.22b	2.21±41.16b	1.82±33.18a
	January	0.05±2.53a	2.54±65.64a	2.84±43.27c	2.13±44.74a	1.93±34.37a
	February	0.08±2.11b	2.77±51.76b	3.02±55.16a	2.18±32.18c	1.81±31.17b
	March	0.08±2.43a	3.02±53.18b	2.81±54.28a	2.10±33.09c	1.78±31.92b
Average		0.07±2.16A	2.68±59.62A	2.56±49.73B	2.47±37.80A	1.78±32.66A

Different lowercase letters indicate significant differences between months, while different uppercase letters indicate significant differences between ages at a significant level (P<0.05).

Table (2) shows the effect of bull age and month on the percentages of progressive and zigzag forward movement and the percentages of live, dead and deformed sperm.

Age groups	Months	Epithets				
		LIN (%)	WOB (%)	Live sperm (%)	Dead sperm (%)	Deformed sperm (%)
3 Years	December	1.56±33.11a	2.82±55.28d	0.73±52.17a	0.76±14.82b	0.42±11.78b
	January	1.72±32.85a	2.93±58.73c	0.56±49.23a	0.39±14.95b	0.38±12.96c
	February	1.78±27.82b	2.87±62.17a	0.82±45.82b	0.32±18.51a	0.61±13.87a
	March	1.67±28.65b	2.74±60.27b	0.74±42.71c	0.45±19.37a	0.67±14.22a
Average		30.61B±1.77	59.11A±2.76	47.48B±0.88	16.91A±0.41	13.21A±0.53
5 Years	December	2.06±37.12a	3.02±47.13d	0.41±61.11a	0.81±13.92b	0.44±9.17c
	January	1.92±34.22b	2.81±50.16c	0.67±57.15a	0.75±14.42b	0.36±11.24b

	February	1.84±30.12d	2.87±57.32a	0.45±53.27c	0.65±15.37a	0.48±12.82a
	March	2.03±32.16c	3.01±54.41b	0.81±48.18d	0.43±16.22a	0.62±13.44a
Average		33.41A±2.05	52.26B±3.11	54.93A±0.74	14.99B±0.79	11.67B±0.51

Different lowercase letters indicate a significant difference between the months, while different uppercase letters indicate significant differences between ages at a significant level (P<0.05).

Table (3) shows that the age of the bulls has a significant effect (P<0.05)in the concentration of total protein, albumin and globulin, as it is noted that the bulls at the age of 5 years significantly outperformed (P<0.05)on the bulls at the age of 3 years in the concentration of total protein, albumin and globulin, as the bulls at the age of 5 years were 3.88, 4.56, 8.44 g / 100 ml and the bulls at the age of 3 years were 3.06, 4.29, 7.35 g / 100 ml respectively, And that this improvement in the average total protein as a result of the aging of animals on the one hand and on the other hand the increase in the capacity of the rumen of the nutrients eaten by the animal all affect the increase in the proportions of protein and albumin in the blood serum of bulls (Mandal et al.,20 10 and Mukhopadhyay et al.,2010), either the reason for the high rates of Globulin in bull serum may be due to the fact that increased levels of globulin are associated with increased immune bodies in the animal's body, which increase with the age of the animal (Waltl et al., 2006, Ahmad et al ., 2011 and Rehman et al., 2012), as can be seen from the same table that the averages of testosterone and FSH It was significantly higher (P<0.05)in the blood serum of bulls aged 5 years compared to bulls aged 3 years, as bulls aged 5 years had 1.76, 1.60 ng/ml, while bulls aged 3 years 1.41, 1.30 ng/ml respectively, and the reason for this difference may be due to the fact that bulls at an older age have Lydic cells responsible for the production of this hormone On the other hand, testosterone is supportive of germ cells (sperm generators) and Sertoli cells, and in turn, the pituitary gland is larger and more developed and secretes FSH Ahmad et al. (2005) and Mulugeta et al., 2006).

It is also clear from Table (3) that the month of the year has a significant effect (0.05 (P< in the biochemical components and hormones in the blood serum of the Iraqi local buffalo bulls, as it is clear in Table (3) that the months of December significantly outperformed (P<0.05)over the rest of the months in the concentration of total protein, albumin and globulin, and for both ages, the age of the bulls was 5 years, 4.98, 5.16, 10.14 g / 100 ml respectively, and for the age of the bulls 3 years were 3.96, 4.77, 8.73 g / 100 ml respectively, and the reason is due to the low temperatures during the month of December for higher equations compared to the rest of the months, which increases the appetite of animals to eat higher amounts of feed and thus raise the rates of protein in the blood, and on the other hand, the low temperatures stimulate the adrenal gland to secrete kaitikol amines, which have an important role in stimulating and producing blood clobulins, and this is confirmed by (Barth and Waldner, 2002 , Ahmad et al., 2005, Bhakat et al., 2009 and Ahmad et al., 2011) in their study of bulls of different ages and seasons. Also from the same table, it is noted that the months of December and January recorded the highest values significantly (P<0.05)in the concentration of testosterone and FSH hormones compared to the rest of the months and not the two ages, so the age of bulls 5 years was 1.85, 2.13 and 1.83, 1.95 ng / ml and for bulls aged 3 years 1.47, 1.64 and 1.37, 1.40 ng / ml respectively, and the reason for this may be attributed to Fluctuation in temperatures during the different months of the year These results were consistent with the findings of (Zamiri et al., 2010 and Khawaskar et al., 2012) who confirmed that the cells of Lidek and their secretions of testosterone *and* vary according to the different months of the year as a result of the variation in temperature.

Table (3) shows the effect of bull age and month on total protein concentrations, albumin, globulin, testosterone andFSH hormones in bull blood serum

Age groups	Months	epithets				
		Total protein (g/100ml)	Albumin (g/100 ml)	Globulin (g/100ml)	Testosterone (ng/ml)	FSH (ng/ml)
3 Years	December	0.27±8.73a	0.13±4.77a	0.12±3.96a	0.03±1.64a	±1.40a0.02
	January	0.21±7.61ab	0.17±4.45ab	0.11±3.16b	±1.47a 0.04	±1.37a0.07
	February	0.18±6.24c	0.11±3.91c	0.28±2.33cd	±1.35ab0.06	±1.22b0.04
	March	0.31±6.82b	0.27±4.02b	0.14±2.80c	±1.17c0.02	±1.18b0.06
Average		±7.35B0.32	±4.29B 0.26	±3.06B0.17	±1.41B0.03	±1.30B0.04
5 Years	December	0.17±10.14a	0.18±5.16a	0.26±4.98a	±2.13a0.06	±1.95a0.03
	January	0.23±8.83b	0.27±4.73ab	0.22±4.10b	±1.85a 0.03	±1.83a0.02
	February	0.11±6.91cd	0.31±4.11cd	0.18±2.80d	±1.62ab0.03	±1.36b0.02
	March	0.13±7.87c	0.22±4.22c	0.20±3.65c	±1.43c0.05	±1.30b0.03
Average		±8.44A0.38	±4.56A0.23	±3.88A0.37	±1.76A0.06	±1.60A0.06

Different lowercase letters indicate a significant difference between the months, while different uppercase letters indicate significant differences between ages at a significant level (0.05(P<.

4. Conclusions:

1- We conclude from the study that the characteristics of the semen of the Iraqi buffalo bulls represented by the individual movement of sperm and its types (VSL, VAP, LIN), sperm concentration, percentage of live sperm and low percentage of dead and deformed sperm improve as the bulls age and that the best ages range between 5 years and more, and the concentrations of total protein, globulins and testosterone hormones Their concentrations at these ages increase in local bulls of Iraqi buffaloes.

2- It is noted that December and January in Iraq are the best months of the year in terms of improving semen traits and this reflects a positive aspect of the increase in fertilization rates and the percentage of pregnancy when inseminated with the sperm of local Iraqi buffalo bulls during these months.

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