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Evaluation Monthly Changes in the Enzymatic Activity, Protein and Semen Characteristics for Holstein Bulls Born in Iraq.

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ABSTRACT

The study was conducted as a first step to recording the modulators of biochemical components (GOT, GPT, ACP, ALP and total protein) in poor and good ejaculation in fresh semen for Holstein bulls born in Iraq, and study monthly variation between these enzyme and total protein in seminal plasma of these bulls. This study was conducted in Artificial insemination center of Abu Ghareeb -Iraq .Semen collected weekly from 5 Holstein bull born in Iraq by using artificial vagina (AV) to evaluate semen properties (ejaculate volume, mass and individual motility, concentration, dead and abnormality percentage of sperms) and also determination the enzymes (GOT, GPT,ACP, ALP) and total protein in seminal plasma of both poor and good ejaculates for all bulls. A result showsthat the respective averages of the transaminases enzymes (GOT more than GPT) and phosphatase (ALP more than ACP) and total protein range between 53.87±4.15—81.58±1.60gram /L , also results shows significant increase ($p< 0.0.5$) in the value of (GOT,GPT,ALP , ACP and total protein) in poor ejaculates in comparison with good , in addition to results revealed that significantly ($p< 0.0.5$) monthly changes in semen characteristicsand enzymes (GOT,GPT,ALP and ACP) values showed significant rise($p< 0.05$) during May in comparison with other months,while there are no significant changes in the value of total protein concentration during different months. In view of this, determination of these biochemical traits is a useful tool to evaluate semen quality and its fitness for development AI in Iraq through uses semen suitable for processing frozen semen.

KEYWORDS enzymatic activity, monthly, semen characteristics, Holstein bulls, Iraq.

Introduction

Seminal plasma is composed of a secretion from the male accessory sex glands and epididymis, which contain many organic and inorganic components that have many effects on semen quality (15). Enzymes and proteins one of these essential components and fundamental knowledge was advanced greatly with regard of study the secretary activity of enzymes and proteins in the seminal plasma using this parameter as a valuable tool to evaluate the fertility potential of the male (4). Transaminase, Glutamic Oxaloacetic transaminase (GOT), Glutamic Pyruvic transaminase (GPT), and phosphatase, Acid phosphatase (ACP), and Alkaline phosphatase (ALP), are essential for metabolic process which provide energy for viability, motility and fertility of spermatozoa (23).In addition to enzymes seminal plasma contain protein, amino acid and polypeptide (9,17,28).These protein have a protective action against the dilution effect by preventing loss of intracellular constituents (17). Similarly, recent reports on seminal plasma proteins described the presence of fertility associated protein in the bovine seminal plasma (8,21).From the many reports it has been found that concentration of transaminas , phosphatase and total protein differ from high to low values according to season, age of animals and also according to fresh ejaculates or diluted one (16), , but Al-Makteri (3) failed to record any significant difference in the activity of ALP of ram semen in and of the breeding season. So the present study will conduct as a first step in a project to study more intensely others related constituents because in the view of this and since little information are available concerning various aspect of the biochemical components (GOT,GPT,ACP,ALP and total protein) in poor and good ejaculation in fresh semen and monthly variation between these enzyme and total protein in semen forHolstein bulls born in Iraq .

Materials &Methods

Bulls

This study was extendedfrom the beginning of December (2014) to the end of May(2015)and carried out at Artificial Insemination Center, Iraq, on **5** Holstein bulls born in Iraq. Semen was routinely collected from all bulls weekly with the aid of an artificial vagina. All bulls were of the same age (4 to 5 years) and were kept under identical conditions of management, feeding and watering throughout the study period. Poor motile ejaculates (estimated less than 50% of individual motility) and good motile ejaculates (estimated equal or more than 50% of individual motility) were taken weekly and assessed as described by (11).

Evaluation of semen quality:

1-Physical properties: A total of 119 ejaculates were studied during period of study. After collection of semen, the sample was immediately brought to the laboratory and placed in a water bath at (37 to 38°C) and a phase-contrast light microscope used for evaluation of individual motility (11). Dead and abnormalities percentages were evaluated using Eosin-Nigrosin stain (9) ,and estimated concentration of spermatozoa by using sperm analyzer .

2- Biochemical evaluation: Immediately after application of few drops of semen needed for various parameters for evalu-

ation of semen quality, 1ml of semen was centrifuged for 30 minute at 4000rpm at 10 C°to separate the seminal plasma , the seminal plasma was checked under microscope to insure that it was sperm free, then it was transferred to sterile vials and stored at -20C°for future analysis of enzymes and protein. Biochemical analysis was performed with an automatic analyzer Tossoh System (Hitachi Bothering Mannheim, 912 Automatic Analyzer) using specific kits to Measured: Enzymes such as, Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT),Alkaline Phosphatase (ALP), Acid Phosphatase (ACP) and Total Protein.

Statistical Analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. T-Test and Duncan (13) multiple range tests were used to determine the significant differences between means in this study. Estimation the correlation coefficients between some parameters have been done .

Results &Discussions

Semen characteristics:

1- Physical properties:

Values of semen volume of poor and good ejaculate is depicted in (tab. 1), the results indicate that semen volume of poor ejaculate on the bulls varied during different months of the year. Highly significant value (p<0.05) was observed during May followed by April while the lowest significant value was in February. Regarding good ejaculate, there is no significant difference between months. On the other hand there is a significant differences (p<0.05) in the ejaculate volume between poor and good ejaculate during the different months of study except February. Values of mass motility of poor and good ejaculates are depicted in (tab. 1). The results indicate that highest values of poor ejaculates was observed in February which is significantly (p<0.05) differed from other months of the year, while the lowest significant values (p<0.05) was observed in May. The values of mass motility of good ejaculate showed highest significant (p<0.05) values was observed in February and the lowest significant (p<0.05) values was in May and December. Regarding the differences between poor and good ejaculate, all the values show significant differences between them (p<0.05) during the different months of the year. Individual motility of Poor ejaculates show highest significant values (p<0.05) was observed in January followed by February and the lowest significant value (p<0.05) was observed in May. Good ejaculate show highest significant value (p<0.05) during December and the lowest significant (p<0.05) values during May and January. Regarding the differences in the individual poor and good ejaculates, all the values show significant differences (p<0.05) except the month

Table (1) Evaluation monthly changes in semen characteristics for poor & good ejaculate of Holstein bulls born in Iraq (Mean ± SE)

Semen characteristics	Bulls	Months					
		December	January	February	March	April	May
Volume (ml)	Poor	6.36 ±0.34 bc	6.20 ±0.43 bc	5.29 ±0.54 c	7.16 ±0.29ab	7.58 ±0.28 a	8.0 ±0.42 a
	Good	4.00 ± 0.42 a	3.80 ±0.59 a	3.62 ±0.54 a	4.56 ±0.77 a	4.25 ±0.50 a	3.88 ±0.2 a
T-test	---	1.38*	1.44 *	1.69 NS	1.48 *	1.32 *	1.19 *
Mass motility (%)	Poor	32.5 ±4.78 abc	39.16 ±4.8 ab	40.88 ±3.8 a	32.50 ±4.87abc	27.5 ±4.2 bc	23.8 ±4.1 c
	Good	52.5 ±1.83 c	55.00 ±2.6 ab	57.50 ±3.1 a	54.37 ±2.2 ab	52.5 ±1.3 c	52.5 ±1.7 c
T-test	---	12.73 *	12.15*	10.20*	12.97 *	11.35*	11.28 *
Individual motility (%)	Poor	38.75 ±4.4 ab	46.66 ±3.6 a	44.16 ±4.4 a	30.58 ±4.2 ab	30.0 ±3.8 bc	22.22 ±4.5 c
	Good	60.0 ±1.3 a	55.62 ±1.4 b	58.12 ±1.8 ab	59.37 ±1.4 ab	58.75 ±1.5 ab	55.0 ±2.5 b
T-test	---	11.86 *	9.82 NS	11.93 *	11.28 *	10.45*	13.04 *
Dead (%)	Poor	24.98 ±2.1 a	18.10 ±2.4 b	24.66 ±2.0 ab	25.25 ±3.6 ab	23.0 ±3.4 ab	25.55 ±2.2 ab
	Good	14.50 ±0.88 c	18.25 ±0.8abc	19.25 ±2.3 abc	19.75 ±1.7 ab	15.12 ±1.4 bc	20.33 ±2.4 a
T-test	---	5.64 *	6.58 NS	6.57 NS	5.23 NS	4.39*	7.21NS
Abnormality (%)	Poor	20.16 ± 1.7 a	16.10 ±1.8 ab	17.62 ±1.8 ab	13.25 ±1.6 bc	10.75 ±1.6 c	13.6 ±1.7 bc
	Good	9.80 ± 0.57 bc	14.33 ±1.1 a	9.75 ± 1.6 bc	7.87 ±1.1 bc	7.00 ±0.7 c	10.83 ±1.2 b
T-test	---	4.53 *	5.14308	5.48 *	4.67 *	4.2258	5.1098
Concentration (*100)	Poor	520.5 ±125.2 b	649.1 ±80.2 b	698.75 ±105.4 b	525.58 ±108.2 b	998.1 ±102.5a	1100.0 ±153.6a
	Good	1490.0 ±203.7 b	1351.6 ±189.4 b	2218.8 ±768.3 b	1637.1 ±188.1 b	1940.1 ±145.6a	1951.0 ±177.1a
T-test	---	471.9 *	382.25 *	1328.2NS	489.6 *	362.6*	513.2*

Within row different small letters for each parametermeans significant at (p<0.05). Within column in each parameter (T Test) * (P<0.05), NS: Non-significant.

of January, as in (tab. 1).Values of dead percentage of poor and good ejaculates is depicted in (table 1).The results indicate that highest values of dead percentage of poor ejaculates was observed in December which is significantly (p<0.05) differed from other months of the year, while the lowest significant values (p<0.05) was observed in January, the values of dead percentage good ejaculate showed highest significant in May (p<0.05) and the lowest significant (p<0.05) values was in April and December. Regarding the differences between poor and good ejaculate, all the values show no significant differences during the different months of the year except December and April. Abnormalities percentage of Poor ejaculates show highest significant value observed in December and the lowest significant value was observed in March and May. Good ejaculate show highest significant value (p<0.05) during January and the lowest significant (p<0.05) values during April is .Regarding the differences in the abnormalities in poor and good ejaculates, all the values show significant differences (p<0.05) in December, February and March, while there is no significant in January, April and May, as in (tab. 1).Values of semen concentration of poor and good ejaculate is depicted in (tab. 1) , the results indicate that semen concentration of poor ejaculate of the bulls varied during different months of the year. Highly significant value (p<0.05) was observed during May followed by April ml while the lowest significant value was in December. Regarding good ejaculate, there are no significant differences between them except in the February. The physical properties of semen of poor and good ejaculates in this study are ejaculate volume, mass activity; individual motility, dead percentage, abnormal percentage and sperm concentration are presented in table (1). These semen properties are testosterone dependent and strongly linked and it would reflect the hormonal profile of the animal (9) the physical characteristics of semen of bulls represent the reproductive performance of the animal which in turn affected by many factors such as environmental factor (ambient temperature, humidity, rainfull), nutrition etc. this agreed with (2,5,25).Values of semen volume of poor and good ejaculates in this study fluctuated during different months of study period and generally speaking the ejaculate volume are found to be in general agreement with the values reported by earlier worker for the bull of the same or different breed that agreed with(6,14,20). but are incontract with the finding of other workers (18).Table (1) show significantly monthly changes in the semen characteristics like mass activity, individual motility, dead and abnormal sperm percentage of good and poor ejaculates as the changes of ejaculate volume which clearly indicated significantly monthly changes in poor ejaculate but no significant monthly changes of good ejaculates. Same thing in sperm concentration the result show significant monthly changes in sperm concentration both in good and poor ejaculate of Holstein bulls of this study. The result in general agreement with the earlier worker (1), but inconstant with other workers (18).Study of good and poor ejaculate of Holstein bull reared in Iraq (table 1) referred to significant difference in ejaculates volume, mass activity, individual motility and sperm concentration which they were significantly high in good ejaculate and low in poor ejaculate, sperm concentration was high in good ejaculate in comparison with poor ejaculate. Atcomparison in the physical characteristics of Holstein bull in AI center of this study (table 1) clearly refers that the worse months on semen quality were the fifth month of year (May) and first month (January) and that is true since these two months characterized by high and low temperature respectively and this acute temperature (high or low) act as a stress factor on the animal resulting in changes in hormonal profile of animal especially testosterone production resulting in deleterious effect on semen quality, this is agreed with (24) .

2- Biochemical properties:

Enzymes:

Values of GOT activity in the seminal plasma comparison of poor and good ejaculates in different months are depicted in (tab. 2) , highest significant difference (p<0.05) in the values

of GOT activity between poor and good ejaculate observed during all the months studied and all the values were highest in poor ejaculate in compression with good ejaculate, regarding the differences in the values of GOT activity during different months in poor ejaculate, highest significant values ($p<0.05$) observed in May and the lowest significant ($p<0.05$) value was in March , and the same thing in good ejaculate, it was significantly high ($p< 0.05$) in May , and lowest value in March . Change of GPT activity in the seminal plasma of poor and good ejaculates in different months are depicted in (tab. 2) , highest significant difference ($p<0.05$) in the values of GPT activity between poor and good ejaculate observed during all the months studied and all the values were highest in poor ejaculate in compression with good ejaculate, regarding the differences in the values of GPT activity during different months, in good ejaculate, highest significant values ($p<0.05$) observed in May followed by January and the lowest significant ($p<0.05$) value was in March and the same trend for poor ejaculate, it was significantly ($p< 0.05$) high in May and lowest values in March .Values ALP activity in the seminal plasma of poor and good ejaculates in different months are depicted in (tab. 2), highest significant difference ($p<0.05$) in the values of ALP activity between poor and good ejaculate observed during all the months studied and all the values were highest in poor ejaculate in compression with good ejaculate, regarding the differences in the values of ALP activity during different months in poor ejaculate, highest significant in May and lowest value in February in comparison with other months and same trend for good ejaculate and the high value in May and low value . Change of ACP activity in the seminal plasma of poor and good ejaculates in different months are depicted in (tab. 2) , highest significant difference ($p<0.05$) in the values of ACP activity between poor and good ejaculate observed during all the months studied and all the values were highest in poor ejaculate in compression with good ejaculate, regarding the differences in the values of ACP activity during different months in poor ejaculate, highest significant values($p<0.05$) observed in May and the lowest significant ($p<0.05$) value was in

Table (2) Evaluation monthly changes in enzymatic activity and total protein for poor&goodejaculate inHolstein bulls born in Iraq
(Mean ± SE

Enzymes	Bulls	Months					
		December	January	February	March	April	May
GOT U/L	Poor	660.6 ±59.7 b	777.5 ±48.2 b	650.0 ±62.3 b	668.5 ±45.4 b	720.0 ±74.2 b	1013.0 ±62.6 a
	Good	470.2 ±54.1 c	549.4 ±52.9 b	476.1 ±41.1 c	462.83 ±35.0 c	572.1 ±50.0 ab	628.8 ±103.4 a
T-test	---	142.5*	172.41*	131.39*	100.12*	192.1*	113.5*
GPT U/L	Poor	78.33 ±7.5 bc	86.00 ±6.4 b	70.66 ±7.9 bc	64.83 ±5.0 c	66.5 ±6.2 c	98.0 ±7.1a
	Good	64.00 ±10.6 b	93.62 ±11.1b	61.87 ±8.1 b	38.75 ±7.8 b	43.12 ±5.6 b	65.50 ±6.6 a
T-test	---	16.47**	15.32 *	14.59 *	12.7**	12.64*	12.01*
ALP KAU/L	Poor	26559.6 ±1433.3 b	21000±2721.8 b	20038.3 ±2429.4 b	23578.3 ±3528.5 b	26400 ±3003.6b	27603.3 ±2641.3a
	Good	12476.2 ±2160.0 c	16657.9 ±2812.1 c	16692.6 ±2309.8 c	12710.7 ±1681.6 b	13047.8 ±2254.8b	17971.9 ±2925.3a
T-test	---	5662.4*	4052.9*	4426.7 *	6050.9*	5982.4*	4317.1*
ACP KAU/L	Poor	1292.5 ±100.6 c	1723.5 ±125.0b b	1276.3 ±197.8 c	1239.5 ±176.0 c	1464.91 ±82.2 b	1974.7 ±188.1 a
	Good	1620.0 ±95.4 b	1818.0 ±230.7 b	1673.1 ±433.4 b	1079.0 ±469.9 b	1355.6 ±188.4 b	1672.3 ±354.5 a
T-test	---	177.2 *	291.9 *	188.1*	194.63*	200.9*	236.1**
Total protein gm/L	Poor	76.16 ±2.5 a	81.50 ±1.6 a	76.66 ±2.92 a	76.16 ±2.7 a	62.41 ±6.3 ab	71.77 ±5.1 ab
	Good	77.75 ±5.66 a	83.87 ±4.0 5 a	78.75 ±5.5 a	75.50 ±7.0 a	57.8 ±6.5 a	62.58 ±15.9 a
T-test	---	11.46 *	8.05 *	11.40 *	13.85 *	10.66 NS	27.4[illegible]

Within row different small letters for each parameter means significant at ($p<0.05$). Within column in each parameter (T Test) * ($P<0.05$),

NS: Non-significant.

January in comparison with other months, and the same thing for good ejaculate, the value was significantly ($p< 0.05$) high in May in comparison with other months. Values total protein activity in the seminal plasma of poor and good ejaculates in different months are depicted in (tab. 2), highest significant difference ($p<0.05$) in the values of total protein activity between poor and good ejaculate observed during all the months studied and all the values were highest in poor ejaculate in comparison with good ejaculate, regarding the differences in thevalues of total protein during different months in poor and good ejaculate, the result indicate no significant difference in the values of total protein concentration in both ejaculate between the different months. Values of Transaminase (GOT and GPT) in the seminal plasma of the Holstein bulls during different months of the study are within the range reported by earlier worker, seminal plasma enzymes studied are known to be involved in several cellular activities (9,19,27). Their presence in the seminal may indicate their source is either intra-cellular (intra spermal) or as a part of the secretions of the accessory sex glands (12). Consequently, are increase in the activities in the seminal plasma may indicate a change in one or both sources. The values of GOT & GPT in the seminal plasma of bull in this study increased significantly during May, the months which is characterized by high ambient temperature in comparison with other studied months which act as thermal stress resulting in changes in the cell membrane integrity of sperm leading to increase sperm membrane permeability to these enzymes (16).Concerning the other two enzymes. Alkaline and Acid phosphatase which are known to be involved in several cellular activities in the male reproductive system, our result in general agreement with the values of (10) but differ from Al-Makteri (3). The results reflect a significant increase in the month of May due to thermal stress which caused sperm damage and increase sperm membrane permeability of these two enzymes. The protein in the seminal plasma has protection action against the dilution effect by preventing loss of intracellular constituents and also presence of fertility associated protein in the bovine seminal plasma (8,21), our result proved no significant change between the months of study.

Conclusion:there is fluctuation in the semen characteristics and Transaminase and phosphatase activity for poor and good ejaculates of Holstein bulls born in Iraq during different months of the year. Transaminase and phosphatase activity besides the total protein in the seminal plasma were highest in the poor ejaculates in comparison with good ejaculates.

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