

## Effect of magnetically treated water on enzymes and total protein in seminal plasma of Holstein bulls born in Iraq

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### Summary

Some enzymes of seminal plasma such as, Glutamic oxaloacetic transaminase, Glutamic Pyruvic transaminase, Acid phosphatase and Alkaline phosphatase are essential for metabolic processes which provide energy for viability, motility and fertility of spermatozoa. These enzymes are used as good indicators of semen quality as they measure the plasma membrane stability of spermatozoa, so the aims of study are to estimate the enzymes level and total protein in seminal plasma of Holstein bulls. In present the available knowledge there is no study in Iraq regarding the effect of using magnetically treated water on enzymes and total protein in seminal plasma of Holstein bulls born in Iraq to improve the Artificial Insemination programs. Sixteen bulls were divided equally into two groups depending on their age. Young (17-19 months) and adult (29-32 months), then randomly distributed into two equal sub groups (control and treatment) through February until June months. Treatment group was given magnetically treated water which passed through magnetic device with strength of 2000 gauss; control group was given normal water without magnetism. Results show that there is a monthly variation in levels of enzymes, in the group drinking magnetic water effect significantly ( $P<0.05$ ), all enzymes of seminal plasma of Holstein bulls in Iraq, with no effect on total protein.

**Keywords: Magnetic water, Enzymes, Total protein, Seminal plasma, Holstein bulls.**

### Introduction

Artificial insemination success basically depends on getting semen of a highly fertile, several enzymes of seminal plasma such as, Glutamic oxaloacetic transaminase (GOT), Glutamic Pyruvic transaminase (GPT), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) are essential for metabolic processes which provide energy for viability, motility and fertility of spermatozoa and these enzymes are used as good indicators of semen quality as they measure the plasma membrane stability of spermatozoa (1). Seminal Plasma contains several proteins, many of which are secretory products of epididymes and seminal vesicles (2). There has been intense research on the identification and functions of seminal plasma proteins in different animals. A comprehensive review on biochemical characteristics and functions of seminal plasma proteins has recently been published by (3), where the Authors have discussed elaborately the protective capability of some low molecular seminal plasma proteins against cold shock of spermatozoa and antioxidant properties. The determination of protein content on sperm

surface and seminal plasma may be an index of individual bull fertilizing ability or post-thaw status of sperm membranes (4). The protein component of seminal plasma varies among species and has important effects on sperm function (5). Many authors believe that the bull seminal plasma contains fertility associated protein markers, and indicated that improving enzymes and protein for seminal plasma of bull it means improving artificial insemination (6). Water and life are closely linked, so many researches study the negative changes in natural water after sterilization and called this water (dead water), so magnetic water transfer water from dead to live (7). Magnetic water increase penetration and flexibility of water (8) and increase absorption of water from body because decrease superficial tying in water (9), surface tension (10), changes in pH (11) and other physical properties such as increase ability of body for production general hormones (12-14) and increase absorption (10). In another hand this changes in magnetic water gives benefits animals such as improved semen characteristics, Fertility rate (15), sexual

hormones (14), enzyme (8 and 16), Blood picture (17), There are no study in Iraq regarding the effect of using magnetically treated water on enzymes and total protein in seminal plasma of Holstein bulls born in Iraq to improvement the Artificial Insemination programs in Iraq. The present study conducted to know if magnetically treated water benefit seminal plasma quality of Holstein bulls born in Iraq, through study transaminas, phosphates enzymes and total protein in seminal plasma and recording the level and monthly variation of these enzymes and total protein in seminal plasma and study comparative between enzymes and total protein in seminal plasma of young and adult Holstein bulls born in Iraq.

### Materials and Methods

This experiment conducted at the Artificial Insemination Center in Abo Ghareeb/Baghdad. Sixteen bulls were divided equally into two groups depending on their age. Young (17-19 months) and adult (29-32 months), then randomly distributed into two equal sub groups (control and treatment), through February until June 2015. Treatment group which drinking magnetically treated water which passed through magnetic device with strength of 2000 gauss, control group drinking normal water. All bulls were kept under identical conditions of management, feeding throughout the study period. Total of 116 and 120 ejaculates from young and adult bulls, respectively, collected by using artificial vagina. Immediately after application of few drops of semen needed for variation parameters for evaluation of semen quality, one ml of semen was centrifuged for 30 minute at 4000 rpm at 10°C to separated seminal plasma (leaking) and checked under microscope to insure that it was sperm free, then it was transferred to sterile vials and stored at -20°C for enzymes and protein analysis. Biochemical analysis was performed with an automatic analyzer Tossoh System (Hitachi Bothing Mannheim, 912 Automatic Analyzer) using specific kits to Measure GOT, GPT, ALP, ACP enzymes and total Protein. The Statistical Analysis System-SAS (18) was used to effect of different factors in study parameters. Duncan multiple range tests were

used to significant compare between means in this study (19).

### Results and Discussion

Effect of magnetically treated water on enzymes: Results indicated that overall means of level enzymes GOT, GPT, ACP and ALP (Tables, 1 and 2), show that enzymes of treated young and adult bulls decreased significantly ( $P<0.05$ ) compared with control bulls during months of April, May and June. The composition, biophysical characteristics and function of extra and intra cellular membranes of spermatozoa might be important factors for releasing these enzymes in seminal fluids of different animals (20). Some researchers report that magnetic water enhanced blood enzymes for Buck (21). The advantage of magnetic water on enzymes of semen might be attribute of positive effects in natural water after magnetic treatment on growing and functions of cells (22 and 23), for example cells which produced milk (24), blood (25) semen (25 and 26), and decrease the damage in sperms of treated bulls comparative with control. Change in abnormal or damaged spermatozoa in semen, levels of enzymes in semen (27). Significantly ( $P<0.05$ ) decreased in enzymes GOT, GPT, ACP and ALP. It means improvement in semen quality (28), ALP enzyme is primary of testicular and epididymal origin could be used as a clinical ejaculatory marker to differentiate azospermia or oligospermia for ejaculatory failure and also means a decrease in membrane plasma damage of sperm, (29 and 30) revealed that a positive correlation between membrane damage and AST, ALT release. Also measurement of these enzymes from seminal plasma is considered to be a sensitive indicator of the plasma membrane damage (31). Results of present study (Table, 1 and 2) showed an absence of the effect of drinking magnetic water on enzymes which might be attributed to short period of expose (from February until March). Enzymes GOT, GPT, ACP and ALP for all bulls increased significantly ( $P<0.05$ ) during June compared with others months might be to increase in temperature during this month, the heat stress produced by the increase environmental temperature negatively affect on semen quality either by its effect on the

mechanical activity which is responsible for maintaining animal body temperature (32) or by its effect on disturbing sperms production and maturation (33 and 34) or by its indirect effect produced from its effect on energy balance in the body (34). June month is take into account among hot months which had a negative effect on quality of semen of bulls (35), thus decreasing the quality of semen causes high concentration of transaminase enzyme in the extra cellular fluid due to sperm membrane damage and easy of leakage of enzymes from spermatozoa (36). The significant, higher levels of phosphatase and transminase in the least freezable ejaculates might reflect initial damage of the spermatozoon membrane, with subsequent increase in its permeability, resulting in leakage of enzymes in the seminal plasma (37).

Effect of magnetically treated water on total protein: in seminal plasma of young and adult Holstein bulls born in Iraq (Table, 1 and 2) revealed that total protein of treated bulls did not different significantly with control bulls except in June month for large bulls, but (21) found in all months increased in total protein of buck semen, might be attribute to different species. (38) Claimed an increase in milk yield of dairy cattle consuming magnetic water without change in milk protein and another's compositions. In spite of (39) reported that magnetic water causes decreased total protein in blood of rats.

The present study determination normal levels of GOT, GPT, ALP, ACP, in seminal plasma of Holstein bulls born in Iraq (Table, 1 and 2). Levels of enzymes GOT, GPT, ALP, ACP were 1530.91 (60.15) U/L, 131.99 (9.36) U/L, 3333.98 (608.84) U/L, 8003.68 (716.06) U/L), (40). The seminal plasma ALP and ACP level reported by (41) as 954.2 U/L and 1268.1 U/L respectively, the level of enzymes in seminal plasma varies based on the level of initial damage to the spermatozoa (30) claimed

that in particular study the semen was not frozen or diluted prior to the sampling for enzyme analysis, in these bulls was in physiological normal range, as it was seen from the physic morphological parameters, making the enzyme level to lie within the normal range. In (Table, 1 and 2) the level of total protein of seminal plasma for young and old bulls. Authors mention that the bovine seminal plasma has protein level of 3 to 8 gm/dL (42), 6.8 gm/dL (43). From above review and present study did not found similarity result, could be attribute to age, species, months, season, etc, which affected on enzymes value and total protein.

Monthly variation in enzymes and total protein in seminal plasma for young and adult bulls (treated and control): (Table, 1 and 2) revealed that monthly variation in enzymes and seminal plasma proteins significant ( $P<0.05$ ) for young and adult bulls, results agree with (6), while (8) reported that enzymes of seminal plasma increased in May month comparative with others months and monthly changes in protein profiles might be due to monthly or seasonal variation in gonado-trophin levels and their receptors in the testes that affect endocrine gonadal function and secretions of epididymes and seminal vesicles, or might occur due to differential protective effect of SP, which might be related with antioxidant enzyme activity (44).

Comparative between enzymes and total protein in seminal plasma for young and adult bulls (treated and control). Results study indicated that overall means of level enzymes GOT, GPT, ACP, ALP and total protein for young bulls (Table, 1) were increased comparative with adult bulls (Table, 2). Positive correlation between concentration of enzymes and age of bulls (45), in addition to the effect of age on level of these enzymes and total protein might be attribute to effect of age on semen characteristics are highly variable between young and adult bulls, (46-48).

**Table, 1: Study effect uses of magnetically treated water on enzymes and total protein in seminal plasma of young Holstein bulls born in Iraq.**

Enzymes	Bulls	Months				
		February	March	April	May	June
GOT (IU/100ml)	Control	880.43±96.25 A a	798.34±66.45 A a	1323.0±100.05 A b	1500.43±88.35 A c	1710.43±68.35 A d
	Treatment	850.32±76.45 A a	772.34±86.11 A a	945.56±111.22 B b	1154.67±99.01 B b	1454.67± 79.51 B c
GPT (IU/100ml)	Control	187.95±36.25 Aa	135.32±29.65 Aa	199.10±48.15 Aa	230.45±36.29 Aa	330.00±46.19 Ab
	Treatment	168.45±46.05 Aa	151.56±46.33 Aa	97.44±56.20 Bb	120.23±46.05 Bb	140.23±39.15 Bc
ACP (IU/100ml)	Control	1780.34 ±100.11 Aa	1561.43±132.44 Aa	1354.44±126.66 Ab	1432.2±116.05 Ab	1189.20±112.33 Ac
	Treatment	1699.23±126.49 Aac	1423.54±119.25 Aa	1103.45±123.99 Bb	1203.54±160.35 Bb	1413.66±100.21 Bc
ALP (IU/100ml)	Control	2204.56±168.29 Aa	2322.00±178.95 Aa	2401.22±196.25 Aa	2445.23±206.11 Aa	4545.21±216.91 Ab
	Treatment	2109.34±167.65 Aa	2412.23±93.12 Aa	1200.56±187.82 Bb	1434.56±188.10 Bb	2197.56±158.66 Bc
Total Protein (mg/ml)	Control	67.21±18.69 Aa	72.89 ± 28.25 Aa	75.43±16.89 Aa	108.45±26.05 Ab	168.01±16.09 Ac
	Treatment	77.23±19.25 Aa	66.70±23.00 Aa	80.56±18.21 Aa	110.23±20.15 Ab	120.11±10.85 Ab

Within row different small letters for each parameter significant at (P<0.05). Within Colum different capital letters for each parameter significant at (P<0.05). Control: drinking water no magnetically. Treatment: drinking water magnetically.

**Table, 2: Study effect uses of magnetically treated water on enzymes and total protein in seminal plasma of adult Holstein bulls born in Iraq**

Enzymes	Bulls	Months				
		February	March	April	May	June
GOT (IU/100ml)	Control	933.22±66.77 A a	878.12±87.15 Aa	1424.0±70.03 A b	1654.43±48.45 A c	1820.08±78.85 A d
	Treatment	877.82±89.85 A	972. 44±63.31 Aa	1005.23±91.45 Bb	1254.22±99.01 Bb	1544.32±69.21 B c
GPT (IU/100ml)	Control	210.05±46.12 Aa	178.67±33.34 Aa	230.00±48.05 Aa	280.45±46.32 Aa	350.00±46.59 Ab
	Treatment	188.85±49.85 Aa	172.56±50. 11 Aa	113.84±46.76 Bb	131.23±56.45 Bb	200.23±39.15 Ba
ACP (IU/100ml)	Control	1922.04±73.54 Aa	1891.12±82.44 Aa	1554.66±96.66 Ab	1462.2±99.75 Ab	1789.20±87.33 Ac
	Treatment	1799.23±89.49 Aac	1679.04±79.25 Aa	1220.45±70.22 Bb	1203.54±81.02 Bb	1513.66±90.21 Bc
ALP (IU/100ml)	Control	2308.86±108.29 Aa	2424.04±138.35 Aa	2471.27±126.35 Aa	2398.87±116.35 Aa	2695.32±116.88 Ab
	Treatment	2294.14±125.78 Aa	2512.66±113.22 Aa	2201.32±117.43 Bb	1887.67±134.89 Bb	2091.56±128.46 Bc
Total Protein (mg/ml)	Control	94.43±10.55 Aa	82.77±18.22 Aa	95.78±12.56 Aa	123.55±20.00 Ab	188.11±16.01 Ac
	Treatment	87.34±13.50 Aa	99.77±14. 03 Aa	80.56±18.21 Aa	130.23±17.15 Ab	120.11±10.85 Bb

Within row different small letters for each parameter significant at (P<0.05). Within Colum different capital letters for each parameter significant at (P<0.05). Control: drinking water no magnetically. Treatment: Drinking water magnetically

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## دراسة تأثير استعمال الماء المعالج مغناطيسياً على الانزيمات والبروتين الكلي للبلازما المنوية لثيران الهولشتاين المولودة في العراق

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### الخلاصة

هناك العديد من الانزيمات في البلازما المنوية مثل، GOT و GPT و ACT و ALP التي تعد ضرورية لعمليات التمثيل الغذائي لتوفير الطاقة ومن ثم حركة النطف وقابليتها على الاخصاب، كما أن تقدير تلك الانزيمات لها دور في تقييم كفاءة نوعية السائل المنوي مثل المحافظة على غشاء النطفة من التلف. إن المعلومات المتوفرة لدينا تدل على عدم وجود دراسات في العراق حول هذا الموضوع لذا فإن الدراسة تهدف إلى معرفة مستويات تلك الانزيمات والبروتين الكلي في البلازما المنوية ومدى تأثير شرب الماء المعالج مغناطيسياً على تلك الانزيمات لما لها من أثر مهم في تطوير برنامج التلقيح الاصطناعي في العراق. أجريت الدراسة على 16 ثور هولشتاين مولود في العراق، قسمت حسب العمر الى مجموعتين متساويتين صغيرة (17-19 شهراً) وكبيرة (29-32 شهراً) كما ان كل مجموعة عمرية قسمت عشوائياً إلى مجموعتين متساويتين وهما مجموعة السيطرة (مجموعة شرب الماء العادي)، ومجموعة المعاملة (مجموعة شرب الماء المعالج مغناطيسياً 2000 غاوس). أظهرت النتائج بأن هناك اختلافاً شهرياً في مستويات الانزيمات، وإن معاملة الماء مغناطيسياً أثرت معنوياً ( $P < 0.05$ ) على جميع الإنزيمات في البلازما المنوية للثيران، لكنه لم يكن له تأثير معنوياً على البروتين الكلي للسائل المنوي لتلك الثيران.

**الكلمات المفتاحية:** الماء المغناطيسي، الانزيمات، البروتين الكلي، البلازما المنوية، ثيران الهولشتاين.