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Research Article

Effect of Dilution, Cooling and Freezing on Physical and Biochemical Properties of Semen for Holstein Bull Born in Iraq

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Abstract | Seminal plasma 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 , study the activity of enzymes such as GOT , GPT, ACP and ALP and proteins in the seminal plasma are essential for metabolic process which provide energy for viability, motility and fertility of spermatozoa in relation to the freezability of semen and using this parameter as a valuable tool to evaluate the fertility potential of the male. The objective of this study was to investigate the effect of different steps of processing frozen semen (dilution, cooling and freezing) on certain physical and biochemical properties of semen for Holstein bull born in Iraq. This study was conducted in Artificial insemination center of Abu Ghareeb (Ministry of Agriculture). Semen collected weekly with the help of artificial vagina (AV) and the ejaculates were evaluated physically and a signed poor and good ejaculates for 5 mature bulls. Results showed a significant effect of dilution, cooling and freezing on the physical and biochemical parameters, with gradual significant rise in GOT, GPT, ALP, ACP and total protein concentration during different stages of dilution, cooling, and freezing of semen and the biochemical properties showed higher significant values in poor ejaculate in comparison with good. Physical properties and biochemical properties affected by different steps of freezing for both poor and good ejaculates.

Keywords | Dilution, Physical and biochemical, Semen, Holstein bull, Iraq

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INTRODUCTION

Artificial Insemination (AI) has the ability to promote quick and broad improvement in genetic quality and opened a new many aspect in animal breeding process through enhancing the selection activity that become a huge economic importance in improving production characteristics of local animals in a short time interval in comparison with natural mating (Bearden et al., 2004). The AI success basically depends on getting semen of a highly fertility, semen consist of seminal plasma and sperms. Seminal plasma 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 (Foxcroft et al., 2008). study the activity of enzymes such as GOT, GPT, ACP and ALP and proteins in the seminal plasma are essential for metabolic process which provide energy for viability, motility and fertility of spermatozoa in relation to the freezability of semen (Pangawkar et al., 1988), and using this parameter as a valuable tool to evaluate the fertility potential of the male (Akusu et al., 1984; Salaam, 2008). Cryopreservation coupled with artificial insemination made rapid strides in genetic improvement of livestock world over during the last millennium. However, the process of freezing and thawing of semen has deleterious effects on post-thaw semen characteristics and fertility (Mutha et al., 2012). Frozen semen in straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle producers. The main factors assumed to be potentially involved in the damage at spermatozoa DNA during the freezing-thawing process are the osmotic stress and the oxidative stress

that occur after thawing (Alvarez and Storey, 1992; Batellier et al., 2000). Longer-term storage of semen is achieved through cryopreservation. It has yet some adverse effect on the spermatozoa manifested as a depression in viability rate, structural integrity, depressed motility and conception rates (Watson, 2000; Batellier et al., 2000; Medeiros et al., 2002). In Iraq several studies proved that the steps of freezing (dilution, cooling, freezing rate and thawing) have an effect on physical properties of semen, but in the view of this and since little information are available concerning various aspect of the biochemical components of the semen of Holstein bulls born and reared in Iraq, the present study will conduct to monitor the changes in the activity of these enzymes during the steps of processing frozen semen.

MATERIALS AND METHODS

Bulls

Ejaculates were collected weekly by artificial vagina from five Holstein bulls born in Iraq age (4-5 year) and maintained at Artificial Insemination Center-Baghdad, the ejaculates which were collected was consider to be poor ejaculate which have individual motility 45-50% and good more than 50%.

Evaluation of Semen Quality

Physical Properties: A total of 43 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 graduated tube used for estimated ejaculate volume , mass and individual motility (Chemineau et al., 1991). Dead and abnormalities percentages were evaluated using Eosin-Nigrosin stain (Bearden et al., 2004).

Biochemical Evolution: Immediately after application of few drops of semen needed for various parameters for evaluation of fresh semen. Two ml of semen divided to two equal samples, 1ml centrifuged for 30 minute at 4000rpm at 10 C°to separate seminal plasma (leaking) and checking this is 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. At the same time the other 1ml of semen sample was diluted by Tris-egg yolk- fructose (dilution rate 1:10), and freezing in liquid nitrogen after package in mini straws by done steps of freezing (dilution, cooling, thawing) and evaluated the physical and biochemical properties of semen during each steps.

Statistical Analysis

The Statistical Analysis System according to program of (SAS, 2012) was used to effect of different factors in study parameters. T-Test and Duncan multiple range tests were used to determine the significant differences between means in this study (Duncan, 1955).

RESULTS

Physical Properties

Results of the study depicted in Table 1 proved a significant decrease (p<0. 05) in the percentage of individual motility of both poor and good ejaculate during the different steps of processing frozen semen in comparison with fresh semen. The study also proved to result in Table 1 significant difference (p<0. 05) in the percentage of individual motility between poor and good ejaculate in all steps of processing the frozen semen.

Table 1: Affect the steps of freezing on semen characteristics for poor & good ejaculate of Holstein bulls born in Iraq (Mean ± SE)

Characters	Quality	Fresh semen	Steps of freezing		
			Dilution	Cooling	Freezing
individual motility (%)	Poor	34. 79 ± 2. 98 a	28. 33 ± 2. 34 ab	26. 67 ± 2. 22 b	25. 62 ± 2. 07 b
	Good	59. 06 ± 1. 04 a	53. 75 ± 1. 25 b	52. 50 ± 1. 44 bc	50. 00 ± 1. 29 c
	T-test	7. 63 *	6. 18 *	6. 02 *	5. 57 *
Dead (%)	Poor	22. 12 ± 1. 09 b	25. 21 ± 1. 40 b	29. 54 ± 0. 95 b	38. 50 ± 1. 62 a
	Good	17. 43 ± 1. 23 b	19. 18 ± 1. 23 b	20. 62 ± 1. 68 b	29. 62 ± 1. 15 a
	T-test	3. 38 *	4. 04 *	3. 65 *	4. 47 *
Abnormality (%)	Poor	12. 00 ± 1. 15 b	13. 21 ± 1. 12 b	10. 95 ± 0. 67 b	20. 91 ± 1. 27 a
	Good	7. 44 ± 0. 63 b	8. 81 ± 0. 67 ab	11. 25 ± 1. 32 a	15. 94 ± 0. 83 a
	T-test	3. 06 *	3. 00 *	2. 74 NS	3. 46 *

Within row different small letters for each parameter means significant at (p<0. 05); Within column in each parameter (**T Test**); ***:** significant (P<0. 05); **NS:** Non-significant

Table 2: Affect the steps of freezing on Enzymes and Total protein in poor & good ejaculate of Holstein bulls born in Iraq (Mean ± SE)

Biochemical properties	Quality	Fresh semen	Steps of freezing		
			Dilution	Cooling	Freezing
GOT U/L	Poor	867. 58 ± 79. 19 c	1641. 96 ± 78. 20 b	1896. 71 ± 78. 69 bc	6794. 08 ± 94. 1 a
	Good	594. 25 ± 89. 84 c	1572. 56 ± 75. 1 bc	2375. 88 ± 63. 7 b	5748. 19 ±75. 8 a
	T-test	117. 51 *	532. 1NS	362. 13*	201. 024*
GPT U/L	Poor	50. 67 ± 4. 11 d	63. 75 ± 2. 34 c	73. 12 ± 5. 59 b	97. 79 ± 10. 61 a
	Good	40. 93 ± 4. 71 b	46. 00 ± 14. 09 b	47. 87 ± 9. 61 b	67. 31 ± 17. 99 a
	T-test	12. 82*	23. 86*	21. 06 *	39. 63*
ALP KAU/L	Poor	22033. 75 ± 788. 7 b	25338. 71 ±750. 4 b	26892. 0 ±821. 4 b	37345. 58 ±892. 2 a
	Good	12883. 31 ± 904. 9 c	13941. 56 ±746. 1 c	15050. 81 ±889. 1 b	18950. 19 ±537. 1 a
	T-test	6582. 0 *	6627. 4*	4053. 3*	2623. 6*
ACP KAU/L	Poor	1050. 21 ± 188. 68 c	1240. 33 ± 173. 1 c	1811. 17 ± 111. 32 b	2772. 13 ±133. 34 a
	Good	962. 81 ±108. 1 b	1006. 88 ± 112. 97 b	1103. 56 ± 100. 96 b	2073. 25 ± 158. 96 a
	T-test	678. 7 NS	4150. 2*	537. 3*	670. 17*
Total protein gm/L	Poor	69. 29 ± 3. 67 b	189. 87 ± 15. 52 b	455. 91 ± 42. 85 a	586. 04 ± 141. 4 a
	Good	56. 25 ± 4. 64 b	374. 31 ± 85. 01 a	574. 871 ± 6. 29 a	541. 43 ± 106. 4 a
	T-test	11. 91 *	144. 85 *	152. 43NS	393. 14NS

Within row different small letters for each parameter significant at (p<0. 05); within column in each parameter (T Test); *: significant (P<0. 05); **NS:** Non-significant

The dead and abnormalities values was depicted in Table 1 proved a significant increase (p<0. 05) for both poor and good ejaculate during the different steps of processing frozen semen, and a significant differences (p< 0. 05) between poor and good ejaculate in percentage of both dead and abnormal sperm had also been recorded.

Biochemical Evaluation

Values of GOT during different steps of freezing are depicted in Table 2, the result indicate a gradual increase in the value of enzyme during the different steps of processing and highest significant value was observed in frozen semen in comparison with fresh semen, diluted and cooled semen. Regarding the differences in values of GOT between poor and good ejaculates, the study proved high significant value (p< 0. 05) was observed in poor ejaculate in comparison with good ejaculate during all steps of processing the frozen semen. Table 2 also shows that values of GPT during different steps of freezing indicate a gradual increase in the value of enzyme during the different steps of processing and highest significant value was observed in frozen semen in comparison with fresh semen, diluted and cooled , regarding the differences in values of GPT between poor and good ejaculate, the study proved high significant value (p< 0. 05) was observed in poor ejaculate in comparison with good ejaculate during all steps of processing the frozen semen. On the other hand ALP enzyme also showed increase during the different steps of processing and highest significant value was observed in frozen semen in comparison with fresh semen, diluted and cooled (Table 2), regarding the differences in values of ALP between poor and good ejaculate, the study proved high significant value (p< 0. 05) was observed in poor ejaculate in comparison with good ejaculate during all steps of processing the frozen semen. In addition to values of ACP during different steps of freezing are depicted in Table 2, the result indicate a gradual increase in the value of enzyme during the different steps of processing and highest significant value was observed in frozen semen in comparison with fresh semen, diluted and cooled, and ACP enzyme high significant (p< 0. 05) in poor ejaculate in comparison with good ejaculate during all steps of processing the frozen semen. Table 2 depicted values of total protein concentration during different steps of freezing, the result indicate a gradual increase in the value of protein during the different steps of processing and highest significant value was observed in frozen semen in comparison with fresh semen diluted and cooled, and total protein high significant (p< 0. 05) in poor ejaculate in comparison with good ejaculate during all steps of processing the frozen semen.

DISCUSSION

Physical Properties

Significant decrease in individual motility and increase in dead and abnormalities percentage for both poor and good ejaculate during different steps, dilution, cooling and freezing of bull semen, this might be attributed to the fact that lactic acid which produced as an end product of sperm metabolism, resulting in harmful lowering of PH which

exerts toxic effect on sperm cell (Ball and Peter, 2004). The considerably reduced values for sperm motility, viability, morphology, and plasma membrane/acrosome integrity observed after cryopreservation of semen over fresh or pre-freeze stage (Chaudhari et al., 2015).

Biochemical Evaluation

Regarding the investigation of transaminases and phosphatases values and concentration of total protein in the seminal of the Holstein bull during different stage of dilution, cooling and freezing of semen proved that there is gradual significant increase in these parameters during the different steps of frozen semen, and that is true since it has been found by earlier worker (Gowda et al., 2010; Sharma et al., 2013). The above mention parameters in the seminal plasma considered as index of sperm damage during dilution, cooling and freezing leading to increase sperm membrane permeability both in good & poor ejaculate but the effect is on poor ejaculate in comparison with good ejaculate (Pangawkar et al., 1988; Szasz et al., 2000; Zedian et al., 2008; Sharma et al., 2013; Srivastava and Kumar, 2014). It appears that spermatozoa damage during storage may be associated with increase sperm membrane permeability and leakage of intracellular enzymes (El-Harairy et al., 2011). The leakage of enzymes from sperm cell into the seminal plasma was very severe. Compared to fresh samples more than 8 fold increases in enzymatic activities of the GOT and GPT and almost 2 folds of ACP and AKP in the frozen-thawed seminal plasma was observed. This increase in enzymatic activity in the frozen-thawed seminal plasma indicates increased cell membrane permeability and damages to the sperm cells during the process of freezing and thawing.

CONCLUSION

Physical properties such as individual motility decrease significantly during dilution, cooling and freezing but percentage of dead and abnormalities and biochemical enzymes and total protein for both poor and good ejaculates increased significant during these steps of freezing.

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AUTHORS' CONTRIBUTION

All authors contributed equally in all the efforts for these articles.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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