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INFLUENCE OFDIFFERENTCONCENTRATION OF CURCUMIN (DIFERUOYL METHANE) ON FROZEN SEMEN QUALITY OF HOLSTEIN BULLS BORN IN IRAQ

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Abstract

Object :The current study was designed to improve semen preserve ability through the use of curcumin (diferuoyl methane) in different concentrations **T1** 0.5 ,**T2** 1.0 , **T3** 1.5 mM/ml and control (without additive) as extender additive expressed in frozen bull semen .

Methods: Ejaculates less than 40% of initial individual motility were taken weekly from (3) Holstein bulls born in Iraq by using the artificial vagina. The semen samples were diluted with Tris containing different concentrations of curcumin (**0.5, 1.0, 1.5**)mM/ml and control (without additive), cooled slowly up to 5 C^{o} and equilibrated for 4hrs, semen was packed into 0.25 ml of straws and freezing and storage 48hrs in liquid nitrogen. Evaluated of semen for individual motility%, dead% ,abnormality%, acrosome integrity% and HOST%.at steps of freezing (after cooling and Post thawing).

Results: Clearly indicated that, addition 1.5 mM of curcumin to tris diluents had significantly ($P<0.05$) higher individual motility%, acrosome integrity% and HOST Test% and less ($P<0.05$) sperm dead%, abnormality %in comparison to the 0.5 Mm of curcumin and control group. Besides, the addition of 1.0 mM of curcumin has also a promising effect when added to the tris-base diluents, while the addition of 0.5 mM of curcumin had their little or no effect on all properties which studies of cooled dilution semen and frozen in liquid nitrogen.in addition to present study revealed that Post-thawing had a clear effect, as it led to a significant ($P<0.05$) decrease in the individual motility%,acrosoma integrity % and HOS Test% of sperm also on other hand caused increased in Dead sperms%, abnormality sperms% compared with after cooling ,but addition curcumin in concentration1.5 mM/ml caused decrease this effect of freezing in some sperms properties such as individual motility% and abnormality% of sperms.

Conclusion: Addition curcumin with concentration 1.5mM/ml To Tris diluent caused improve poor sperms properties of bulls and decrease effect of freezing on these samples.

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Keywords: curcumin, frozen semen, Holstein bulls, Iraq

INTRODUCTION

Redox biology implies a small increase of the reactive oxygen species(ROS) level to activate signaling pathways, but oxidative stress involves elevated ROS levels, resulting in the impairment of cellular nucleic acids, proteins or lipids (Schieberand Chandel, 2014). It is well documented that ROS formed by the univalent reduction of oxygen(Bilodeauet al, 2000) .Therefore, the balance between ROS generation and antioxidants should be precise for the protection of spermatozoa (Iqbal et al, 2016 b).Antioxidants are generally defined as compounds with slow autoxidation(Li&Pratt, 2015) ,and antioxidant reduced oxidative stress – induced by hydrogen peroxide (Alwan and Al-Okialy, 2018). Curcumin (diferuoyl methane), a natural RTA, is a yellow compound isolated from the dried ground rhizomes of the perennial herb Curcuma longaL. of the family Zingiberaceae. The conversion of 1, 3-dicarbonylmoiety of curcumin to an isosterichetero cycle as in pyrazolecurcumin, which decreases its rotational freedom, leads to an improvement of its redox properties as well as its antioxidant activity (Jha, 2015). During cryopreservation, there is an imbalance between the ROS and the natural antioxidants resulting in oxidative stress. Therefore, to minimize the effects of oxidative stress on spermatozoa during cryopreservation, addition of antioxidants in semen extender is required(Andrabi, 2008). Outcomes of spermatozoa cryopreservation with curcumin in extender have been reported in Angora buck (Bucak et al., 2010), Holstein bull (Bucak et al., 2012), miniature boar (Jeon&Kim, 2013) , albino Wistar rats (Soleimanzadeh&Saberivand, 2013) and buffalo (Shah et al,2016). Moreover, oral administration of curcumin have been reported to improve the morphologic features of spermatozoa in New Zealand White rabbit buck (Seadawy, 2014) and mice (Mathuria&Verma, 2008; Głombik et al.,2014; Lin et al., 2015). In spite of many researches in world used curcumin (diferuoyl methane) as antioxidant in extender of semen but in Iraq no found any study about this, especially for improve production frozen semen by Artificial insemination (A.I) center –Baghdad. Therefore this study was aimed to investigate the effect of different concentrations of Curcumin as antioxidant on semen quality during different steps of semen processing (cooling , freezing).

Materials and Methods

Semen collection and initial evaluation

This study was carried out at the Artificial Insemination Center, Abou-Ghareeb Western of Baghdad, on (3) three Holstein bulls born in Iraq, all bulls age (3-4year) old and they were kept under identical conditions of management, feeding and watering. Semen was collected from bulls weekly with the aid of an artificial vagina method, immediately after collection, semen placed in water bath at 37ºC until their assessment in the laboratory, ejaculates of semen with less than (40) percent initial motility was used for the trail.

Semen processing

Semen samples were allocated to four fractions and diluted to a final concentration of 30 X 106 spermatozoa/straw (set by using photometer system used in AI center) with the base diluter Tris-citric acid containing curcumin 0.5mM/ml, 1.0mM/ml, 1.5mM/ml), and Tris-citric acid control (without curcumin). Semen was cooled to 4°C in2-hr ,then subjected to 4-hr equilibration. Cooled semen samples were loaded into 0.25 ml French straws and frozen in a programmable which used by Artificial Insemination Center in Iraq.

Semen quality assessment

These assessments were undertaken on fresh semen, after cooling and post- thawing of bull spermatozoa, frozen straws were thawed at 37ºC for 30 seconds in a water bath for evaluation, the parameter studies were the sperm and evaluated the individual motility% (Chemineauet al., 1991), dead % (Blom 1950), abnormalities% (Evans and Maxwell, 1987), Acrosoma integrity % (Kovács and Foote 1992,Kumar et al 2018) and HOST Test% (Jeyendran et al 1984, Hishinuma and Sekine 2003).

Statistical analysis

The experiment was conducted. Results are quoted as Mean ± SE. Statistical analyses were carried out using the General Linear Model procedures (GLM) of SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) .Comparisons between values were analyzed by Duncan's multiple range test following an F-test in ANOVA (Duncan. 1955). Significance was set at ($P<0.05$).

Results

1-Influence of different concentration of curcumin on sperms properties oF Holstein bulls born in Iraq:

1- Individual motility %

Present study which show in Table 1.Revealed that After cooling diluted semen with addition curcumin at a concentration T3 (39.44±1.134) and T2 (38.33±1.895) caused a significant

(P<0.05) increase in the individual motility % of sperm compared to the concentrations T1 (33.33±1.021) and control (33.88 ±1.594). While no significant differences appeared between control and addition curcumin at a concentration T1 and among T2 with T3 .

Table (1).Effect different concentration of curcumin on individual motility% of sperms for Holstein bulls born in Iraq during different steps of freezing (Mean ±SE) .

concentration of curcumin mM/ml	Steps of freezing		Overall mean
	After cooling	Post-thawing	
Control	33.88 ±1.594 Ba	26.50±1.033 Bb	30.25±1.375 B
T1 = 0.5	33.33±1.021 Ba	26.69±1.313 Bb	29.89±1.565 B
T2 = 1	38.33±1.895 Aa	31.50±1.500 Ab	34.89±1.456 A
T3 = 1.5	39.44±1.134 Aa	35.00±1.632 Aa	37.01±1.477 A
Overall mean	35.59±0.920 a	29.03±0.969 b	

Within column different large letters for each parameter and each month differed significantly (p<0.05).

Within row different small letters for each parameter and each month differed significantly (p<0.05).

As shown in Table 1 during post-thawing, the addition of curcumin by concentration T2 (31.50±1.500) and T3 (35.00±1.632) led to a significant (P<0.05) increase in individual motility% for sperm compared with T1 (26.69±1.313) and control(26.50±1.033), but no significant differences emerged between the T2 and T3, as well as between T1 and control. Overall mean of individual motility% of sperms in T3 (37.01±1.477) and T2 (34.89±1.456) increase significant (P<0.05) compared to the T1 (29.89±1.565) and the control (30.25±1.375) but no significant differences **emerged between the T2 and T3 as well as between T1 and control (Table 1).**

2- dead %

It is clear from Table 2. After cooling diluted semen with addition curcumin at a concentration T2 (30.53±0.109) and T3 (26.52±0.225) caused a significant (P<0.05) decrease in the dead percentage of sperm compared to the concentrations T1 (46.80±0.271) and control

(47.11±0.188),in addition to T3 it had a role in lowering significant (P<0.05) in dead% compare with T2 While no significant differences appeared between control and addition curcumin at a concentration T1.

Table (2).Effect different concentration of curcumin on Dead % of sperms for Holstein bulls born in Iraq duringdifferent steps of freezing . (Mean ±SE)

concentration of curcumin mM/ml	Steps of freezing		Overall mean
	After cooling	Post-thawing	
Control	47.11±0.188 Ab	55.20±1.658 Aa	51.57±1.760 A
T1 = 0.5	46.80±0.271 Ab	56.80±1.583 Aa	50.23±1.799 A
T2 = 1	30.53±0.109 Bb	35.95±1.80 Ba	33.17±1.661 B
T3 = 1.5	26.52±0.225 Cb	35.60±2.008 Ba	35.89±1.605 B
Overall mean	37.48±0.858 b	45.98±1.084 a	

Within columndifferent large letters for each parameter and each month differed significantly (p<0.05).

Within rowdifferent small letters for each parameter and each month differed significantly (p<0.05).

As shown in Table 2 during post-thawing, the addition of curcumin by concentration T2 (35.95±1.80) and T3 (35.60±2.008) led to a significant (P<0.05) decrease in dead % sperm compared with T1 (56.80±1.583) and control(55.20±1.658), but no significant differences emerged between the T2 and T3, as well as between T1 and control. Present study of overall mean for dead sperms % in T3 (35.89±1.605) and T2 (33.17±1.661) decrease significant (P<0.05) compared to the T1 (50.23±1.799) and the control (51.57±1.760) but no significant differences emerged between the T2 and T3 as well as between T1 and control (Table 2).

3-Abnormality %

The result of effect different concentration of curcumin on abnormality sperms % (Table 3), found during After cooling of diluted semen at a concentration curcumin T1 (11.19±0.243) , T2 (10.08±0.092) and T3 (9.05±1.037) caused a significant (P<0.05) decrease in the this properties compared to the control (16.44±1.070). While no significant differences appeared

Table (3).Effect different concentration of curcumin on Abnormality % of sperms for Holstein bulls born in Iraq during different steps of freezing . (Mean ±SE)

concentration of curcumin mM/ml	Steps of freezing		Overall mean
	After cooling	Post-thawing	
Control	16.44±1.070 Ab	21.65±0.046 Aa	19.30±0.908 A
T1 = 0.5	11.19±0.243 Bb	18.80±0.983 Aa	15.19±0.004 B
T2 = 1	10.08±0.092 Bb	14.80±0.132 Ba	12.18±0.913 C
T3 = 1.5	9.05±1.037 Ba	12.10±0.1003 Ba	10.85±0.892 C
Overall mean	11.75±0.489 b	16.44±0.557 a	

Within columndifferent large letters for each parameter and each month differed significantly ($p<0.05$).

Within rowdifferent small letters for each parameter and each month differed significantly ($p<0.05$).

between all treatments T1,T2 and T3 . As shown in Table 3 during post-thawing, the addition of curcumin by concentration T2 (14.80±0.132) and T3 (12.10±0.1003) led to a significant ($P<0.05$) decrease in Abnormality percentage for sperm compared with T1 (18.80±0.983) and control(21.65±0.046), but no significant differences emerged between the T2 and T3, as well as between T1 and control. Results of overall mean for abnormality percentage of sperms in T3 (10.85±0.892) and T2 (12.18±0.913) decrease significant ($P<0.05$) compared to the T1 (15.19±0.004) and the control (19.30±0.908) and also T1 give less significant ($P<0.05$) compared with control but no significant differences emerged between the T2 and T3 (Table 3).

4-Acrosoma %

Acrosoma integrity % of diluted sperms during after cooling which is proven inTable 4. Revealed that addition curcumin at a concentration T2 (75.77±1.186) and T3 (78.52±1.106) caused a significant ($P<0.05$) increase in the acrosoma integrity% of sperm compared to the concentrations T1 (70.91±1.168) and control (72.36±1.120). While no significant differences

appeared between control and addition curcumin at a concentration T1 and among T2 with T3 . During post-thawing as shown in Table 4., the addition of curcumin

Table (4).Effect different concentration of curcumin on Acrosoma integrity % of sperms for Holstein bulls born in Iraq during different steps of freezing (Mean ±SE).

concentration of curcumin mM/ml	Steps of freezing		Overall mean
	After cooling	Post-thawing	
Control	72.36±1.120 Ba	50.60±1.550 Cb	61.16±1.403 D
T1 = 0.5	70.91±1.168 Ba	51.05±1.309 Cb	65.39±1.258 C
T2 = 1	75.77±1.186 Aa	63.10±1.942 Bb	69.76±1.410 B
T3 = 1.5	78.52±1.106 Aa	68.55±1.613 Ab	73.96±1.285 A
Overall mean	74.10±0.675 a	55.64±0.564 b	

Within column different large letters for each parameter and each month differed significantly ($p<0.05$).

Within row different small letters for each parameter and each month differed significantly ($p<0.05$).

by concentration T3 (68.55±1.613) led to a significant ($P<0.05$) effect in maintenance acrosoma integrity% compared with T1 (51.05±1.309), T2 (63.10±1.942) and control(50.60±1.550), and T2 better significant ($P<0.05$) than T1 and control but no significant differences emerged between T1 and control. It was noted that the overall mean of acrosoma integrity% of sperms in all treatments T1 (65.39±1.258) , T2 (69.76±1.410), and T3 (73.96±1.285) showed positive significant ($P<0.05$) compared with control (61.16±1.403) and better safety acrosoma integrity% found in T3 among others treatments T1 and T2 (Table 4).

5-HOST Test %

Plasma membrane integrity (HOST Test %) of diluted semen was presented in Table 5.which showed that this parameter after cooling the treatment T3 (71.36±0.687) is better significant ($P<0.05$) than the treatment T2 (67.16±1.672), but at the same time, both are better significant ($P<0.05$) than T1 (60.22±0.557) and control (60.33±0.088) While no significant differences appeared between control and addition curcumin at a concentration T1 . HOST Test % for

post-thawing of frozen bull semen was present in Table 5. Explained that T3 (64.55±0.013) give more a significant (P<0.05) of HOST Test % for sperms were compared with T1 (58.95±0.021) and control(57.55±0.318), and T2 better significant (P<0.05) than control but no significant differences emerged between T2 compare with T1 and T3 ,as well as the different no significant between T1 and control (Table 5.) , from this table noted that the overall mean of HOST Test % of sperms in T3 (67.92±0.424) best significant (P<0.05) compared to the T2 (64.26±0.334) were both more significant (P<0.05) than T1 (59.26±0.431) and the control (58.55±0.371) but no significant differences emerged between T1 and control .

Table (5).Effect different concentration of curcumin on HOST Test% of sperms for Holstein bulls born in Iraq during different steps of freezing. (Mean ±SE).

concentration of curcumin mM/ml	Steps of freezing		Overall mean
	After cooling	Post-thawing	
Control	60.33±0.088 Ca	57.55±0.318 Ca	58.55±0.371 C
T1 = 0.5	60.22±0.557 Ca	58.95±0.021 BCa	59.26±0.431 C
T2 = 1	67.16±1.672 Ba	61.85±0.538 ABb	64.26±0.334 B
T3 = 1.5	71.36±0.687 Aa	64.55±0.013 Ab	67.92±0.424 A
Overall mean	64.42±0.923 a	60.37±0.823 b	

Within column different large letters for each parameter and each month differed significantly (p<0.05).

Within row different small letters for each parameter and each month differed significantly (p<0.05).

2-Effect steps of freezing on some semen properties of Holstein bulls born in Iraq:

It is clear from the results in Tables 1,2,3,4,5 which illustrate the effect of the freezing steps (after cooling and post-thawing) in the tris (control) and the added to it different concentrations of curcumin on the percentage of semen characteristics which are:

1-Individual motility %: It was observed in Table 1, that the post-thawing had a clear effect, as it led to a significant (P<0.05) decrease in the individual motility of sperm% compared to

that motility after cooling for both the control (26.50±1.033 ,33.88 ±1.594) , T1 (26.69±1.313 ,33.33±1.021) and T2 (31.50±1.500,38.33±1.895), but post-thawing did not effect on individual motility of sperm% in T3 (35.00±1.632 ,39.44±1.134), on otherwise appear the different significant (P<0.05) between the overall mean of the individual motility after cooling (35.59±0.920) and post- thawing (29.03±0.969).

2- Dead %: As shown in Table 2 .dead sperms % through post-thawing had a clear increase a significant (P<0.05) which compare with after cooling in control (55.20±1.658 , 47.11±0.188) , T1 (56.80±1.583 ,46.80±0. 271) T2 (35.95±1.80,30.50±0.109) and, T3 (35.60±2.008 ,26.52±0.225), in addition to overall mean of the dead sperms % post- thawing (45.98±1.084) more significant (P<0.05) than after cooling (37.48±0.858).

3-Abnormality %: The mean incidence of post-thaw abnormality sperms% in bull semen samples (Table 3)revealed that frozen semen effect significant (P<0.05) on this parameter of sperms where found in after cooling the control (16.44±1.070) , T1 (11.19±0.243) and T2 (10.08±0.092) were less than post-thawing for control (21.65±0.046), T1(18.80±0.983) and T2(14.80±0.132) but frozen semen did not effect on abnormality sperms% for semen which diluted with T3 where it was no significant between after cooling(9.05±1.037) and post – thawing(12.10±0.1003),but overall mean observed that the different between these steps of freezing were significant (P<0.05) it can noted that abnormality sperms% in post-thawing (16.44±0.557) rise significant (P<0.05) compare with after cooling (11.75±0.489) .

4-Acrosoma integrity %: Comparison of post-thawing and after cooling for acrosoma integrity % of bull sperms (Table 4), the different between them were significant (P<0.05) whether for control(50.60±1.550,72.36±1.120),T1(51.05±1.309,70.91±1.168),T2 (63.10±1.942,75.77±1.186) and T3 (68.55±1.613 ,78.52±1.106)respectively, on otherwise appear the different significant (P<0.05) between the overall mean of the Acrosoma integrity of sperm after cooling (74.10±0.675) and post- thawing (55.64±0.564).

5-HOS Test %It was observed in Table 5 that the post-thawing had a clear effect, as it led to a significant (P<0.05) decrease in the HOS Test% (plasma membrane integrity of sperms) compared to after cooling for both T2 (61.85±0.538,67.16±1.672) and T3 (64.55±0.013 ,71.36±0.687) respectively, but steps of freezing did not effect on plasma membrane integrity (HOS Test) of sperms % for the control (57.55±0.318,60.33±0.088) and T1(58.95±0.021,60.22±0.557), on otherwise appear the different significant (P<0.05) between the overall mean of the HOS Test of sperm after cooling (64.42±0.923) and post- thawing (60.37±0.823).

DISCUSSION

1- Influence of different concentration of curcumin on sperms properties of Holstein bulls born in Iraq.Present study which show in Table1.Revealed that during After cooling and post-thawing diluted semen with addition curcumin at a concentration 1.0 and 1.5 mM/ml caused a significant ($P<0.05$) increase in the individual motility % of sperm compared to the concentrations 0.5 and control . Our results agree with Shah et al 2016 show that at pre-freezing and post- thawing, we found higher seminal total antioxidant contents with 1.5 and 2.0 mM concentrations of curcumin in extender compared to 0.5 and 1.0 mM of curcumin and control. This signifies that curcumin added at the concentrations of 1.5 and 2.0 mM in cryodiluents were able to alleviate the total seminal antioxidant contents during the cooling and freezing processes, and the same result for goat semen they reached by Bucak et al. (2010) in that an addition of curcumin could improve progressive motility Angora goat semen and found improve the progressive motility and functional integrity of the sperm plasma membrane of frozen bull semen (Tvrdá et al 2015). Also for ram semen motility parameters recorded by our CASA system are in accordance with previous findings by Bucak et al. (2008; 2010) demonstrating a marked improvement in the motion of post-thawed ram spermatozoa supplemented with the curcumin. For this reason show that curcumin decrease the negative effect of freezing. Freezing of the sperm causes cold shock during the freezing process, damage due to phase change in membrane structures and oxidative stress. Developing oxidative stress and cytotoxic aldehydes (malondialdehyde etc.) cause damage to spermatozoon functions (Aitken, 1994). For this reason, cold shock damage in the environment can be minimized by adding some cryoprotective and antioxidative additives to semen diluents. The fact that antioxidant compounds also have cryoprotectant properties provides better results from semen frozen with these substances (Alvarez et al 1987,Kobayashi et al 1991).Our results correlate with the report by Soleimanzadeh and Saberivand (2013) in frozen-thawed semen, where curcumin addition had a positive impact on both motility and viability. One of the possible ameliorative mechanisms of curcumin on the above mentioned parameters is to scavenge the free radicals and thereby act as an antioxidant. Another reason for the enhancement of sperm motility in spermatozoa observed in this study may be due to the increasing levels ROS(reactive oxygen species) scavenging molecules, being in accordance with total antioxidant level of semen in curcumin supplemented group by (Soleimanzadeh and Saberivand 2013). Physiologically relevant concentrations of ROS have been shown to participate in maintaining optimal membrane stability, acrosome integrity, and fertilizing potential of mammalian spermatozoa (Sikka,2004). It is well known that there is an

overproduction of ROS because of oxidative stress during semen processing and cryopreservation (Andrabi, 2007). Addition of antioxidants in semen extender controls the degree of ROS generation during cryopreservation (Iqbal et al., 2016a, b).The lipid peroxidation cascade is initiated with the attack of ROS on polyunsaturated fatty acids of the sperm plasmalemma (Baumber et al, 2003). The susceptibility of mammalian spermatozoa to oxidative stress is because of their higher concentrations of unsaturated fatty acids and of limited repair mechanisms (Van Loon et al., 1991; Andrabi, 2009). Moreover, it has been reported that the seminal antioxidant content is inadequate for the prevention of lipid peroxidation during freezing and thawing process (Storey, 1997). Consequently, fortification of cryo diluent with antioxidant such as curcumin is required (Andrabi et al., 2008).Present now results of overall mean in Table 3 for abnormality percentage of sperms in T3 and T2 decrease significant ($P<0.05$) compared to the T1 and the control. With regard to the biphasic effect on the concentration of curcumin in the present study, it is in agreement with previous reports in that the qualities of frozen-thawed boar semen depend on the concentration of antioxidants (Chanapiwat et al. 2009; Rithaporn et al. 2003; Naz 2011,Salahshoor et al. 2012) .The first report on the useful effects of curcumin (Bucak et al., 2008) on post thawed sperm morphology and antioxidant activities of cooled ram spermatozoa has given rise to its use as an antioxidant additive and has been shown to have cryoprotective effects when added to the freezing extender. In addition to the our result about effect of addition curcumin on acrosoma integrity% and HOST Test % which present in Table 4 and Table 5.show that that addition curcumin at a concentration T2 and T3 caused a significant ($P<0.05$) increase in the acrosoma integrity% of sperm compared to the concentrations T1 and control (Table 4),and also found the treatment T3 is better significant ($P<0.05$) than the treatment T2 , but at the same time, both are better acrosoma integrity% significant ($P<0.05$) than T1 and control (Table 5). Our results agree with (Tvrdá et al 2015) in addition to Bucak et al. (2010) reported that an addition of curcumin could improve acrosome integrity of frozen-thawed Angora goat semen and also improve integrity of the sperm plasma membrane of frozen bull semen. The integrity of sperm plasma membrane and acrosome is an essential criterion to sustain spermatozoal functions in the female's reproductive tract and oocyte penetration (Holt, 2000; Esteves et al 2007). At post- thawing, we found higher supra vital plasma membrane integrity and percentage of viable spermatozoa with intact acrosome by adding 1.5 mMcurcumin in extender compared to 2.0 mM dose and control. These findings signify that 1.5 mMcurcumin in cryodiluent acted as a membrane stabilizing antioxidant by preventing the elevation of lipid peroxidation level. However in the present study, the lipid peroxidation levels were similar in

extenders containing 1.5 and 2.0 mMcurcumin; as narrated above, this discrepancy could be due to the toxic effects of curcumin at dose level of 2.0 mM (Naz, 2011). It is worth to mention that in our study at post- thawing, a significant association between supra vital plasma membrane integrity and sperm progressive motility/rapid velocity was found. Regarding acrosome integrity, Omur and Coyan (2016) reported that it was higher with 1, 2 and 4 mMcurcumin in Merino ram. Bucak et al. (2012) reported no significant differences in bovine sperm acrosome abnormalities with 0.5 mMcurcumin in extender. The possible reason for low efficacy of curcumin at 0.5 could be its insufficient concentrations in creating redox homoeostasis as it was observed during the seminal total antioxidant capacity and lipid peroxidation evaluation (Shah et al 2016). Because of this characteristic, curcumin can protect the plasma membrane against lipid peroxidation. We determined that membrane integrity was preserved by 1 and 2mM/ml curcumin when compared to the control and other antioxidant groups. All antioxidant groups exhibited positive effects in terms of acrosomal status and motility in comparison to the control. and exerted protective effects with respect to functional integrity of the membrane in comparison to the control in experiments of freeze-thawed bovine semen (Bucak et al. 2012). From these results of authors , in present study we used the concentration 1 and 1.5Mm/ml if the best ,may therefore speculate that CUR has the ability to exhibit a dual biological activity: while mount of CUR concentrations range among 1-1.5Mm/ml may protect and stimulate the activity of male reproductive cells, higher CUR doses may exhibit toxic effects on the sperm vitality. As such, the exact critical dosage of CUR may be still an important issue to be studied in detail, as it may be affected by the semen processing protocol, time of exposure or animal species (Naz 2011,Bucak et al.2010, 2012),but Millan et al 2017 show that adding 2-3mM/ml curcumin can have favorable effects on post-thawed rooster sperm motility parameters, viability, plasma membrane integrity, and abnormality ,may be attributed to the breed of male affected by concentration of curcumin, where Lee et al 2017 found 0.5mM/ml curcumin improved sperm characteristics such as motility, viability, mitochondrial activity, and plasma membrane integrity, and may exert a positive effect on sperm fertility in pigs. Conversely, it was reported that curcumin did not fully contain the lipid peroxidation in bull and goat spermatozoa at post- thawing (Bucak et al., 2010, 2012). So this discrepancy could be attributed to the species differences (water buffalo versus buck and Taurus bull) in response to the doses of curcumin in cryo diluents.

2-Effect Steps of freezing on sperm properties (individual motility, dead , abnormality, acrosome integrity and HOST%) after dilution with Tris diluent and addition different concentration of curcumin .

The result in present study were concluded in Table 1,2,3,4,5 and clear that cooling and post-thawing effect significant($P < 0.05$) on sperms properties included individual motility %, dead%, abnormalities% , acrosome defects% and HOST Test % in Table 1, 2, 3, 4 and 5., these are show that steps of freezing effected significant on characteristics of sperms ,what is reached is compatible with (Martínez&Pardo, 2013; Al-Badrany et al , 2017; AL-Badry et al, 2016). The fact that progressive motility is more affected by the freezing process than individual motility implies that these parameters measure different aspects of cell physiology and in particular, that the physiological basis for the progressive motility parameter is more sensitive to cryobiological damage (Anel et al , 2003). During cryopreservation spermatozoal mitochondria undergo damages (Gillan et al , 2004; Peris et al , 2004). Üstuner et al , 2015 explain that the freezing process negatively affects ($P < 0.05$) the sperm parameters (individual motility, dead and abnormality), agreement with . (Üstuner et al , 2015). Hussain et al (2016) reported that 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 & 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), but when referring to the results shown in the tables above, we notice that the studied characteristics, whether in Tris diluent control or addition different concentration of curcumin, are more rich in post thawing compared to after cooling, but this effect is less when adding 1.5mM/ml curcumin it had a role in protecting sperms from effect freezing, where it was observed that there was no significant decrease in the individual motility of sperm% (Table 1) and it also appeared that the attributes were mention above and dead% (Table 2) ,acrosoms integrity % (Table 4) and HOST Test % (Table 5), were less affected by freezing compared to 0.5 mM/ml and control. The results of the current experience regarding this aspect summarized that addition curcumin to Tris diluent especially concentration 1 and 1.5Mm/ml improve freezability of bull sperms .Successful sperm storage (liquid and frozen) requires slowing of the cell metabolism and thereby prolongs viability (Maxwell and Salamon, 1993; Gibb and Aitken, 2016). and the concentration of the antioxidants present in the semen can be reduced by dilution, and as a result decreasing the beneficial effect of this endogenous antioxidative defense. Thus, the addition of antioxidants, even in small concentrations, can improve sperm function during preservation.Frozen semen production consisted of a series of the process

including semen dilution in extender, freezing, cold storage and post-thawing evaluation and freezing/thawing of sperm sample is routinely conducted to perform artificial insemination all these procedures (cryopreservation) produce reactive oxygen species (ROS) or free radicals in sperm samples (Saraswat et al. 2016)so during cryopreservation, semen is exposed to cold shock and atmospheric oxygen, which increases their susceptibility to lipid peroxidation (LPO) and moreover excessive production of these reactive oxygen species (ROS), impairs the motility and fertilization capacity (Saraswat et al. 2013).Exposure of spermatozoa to low temperatures has been repeatedly associated with adenosine triphosphate depletion, premature capacitation, and acrosome reaction as well as increased morphological alterations (Ball 2008). At the same time, it must be emphasized that a proper sperm motility, intact membranes and mitochondria are essential to enable the sperm transit through the cervical structures followed by their penetration through the cumular cells and zonapellucida of the oocyte. In this study, from the present study show that CUR in concentration 1.5 mM/ml behaved as an efficient promoter of sperm motility and a membrane-protecting agent enhancing the sperm structural integrity following freezing and thawing, leading to a significant maintenance of sperm motility characteristics.

Increase ROS production by weakening spermatozoa can have detrimental effect on sperm function (Baumber et al., 2000) Moreover, freeze-thaw cycle reduces the level of antioxidant in mammalian semen (Stradaioli et al., 2007) and therefore caused negatively effect on the viability of sperm so the presence of turmeric in these concentration , The addition of antioxidants to the freezing extender (Bilodeau et al., 2001; Uysal et al., 2007; Sarıo¨zkan et al., 2009) has been shown to protect spermatozoids against the harmful effects of ROS and to improve post-thaw sperm functions such as motility, viability and fertility. Curcumin has been subjected in many researches regarding various cell systems against ROS-induced damage and organ transplantation and storage due to its highly efficient cryoprotective and antioxidative properties against cold shock and oxidative damage (Abuarqoub et al., 2007; Kanitkar&Bhonde, 2008; Mathuria&Verma, 2008). Curcumin is an antioxidant act as beneficial molecules on sperm cells by neutralizing H2O2 and inhibiting reaction with free radicals (Ugur et al 2019). On other hand concentration 0.5 mM/ml do not play this role may be the possible reason for low efficacy of curcumin at 0.5 mM/ml could be its insufficient concentrations in creating redox homoeostasis as it was observed during the seminal total antioxidant capacity and lipid peroxidation evaluation (Shah et al 2016).

CONCLUSION

Addition Curcumin at concentrations 1.5 mM/ml better significant than 1mM/ml were both caused improve preservation poor motility of bull sperms compare with 0.5Mm/ml and control. Also present study revealed that Post-thawing had a clear effect, as it led to a significant ($P<0.05$) decrease in the individual motility%, acrosoma integrity % and HOS Test% of sperm also on other hand caused increased in Dead sperms%, abnormality sperms% compared with after cooling ,but addition curcumin in concentration1.5 mM/ml caused decrease this effect of freezing in some sperms properties such as individual motility% and abnormality% of sperms.

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