EFFECTS OF ZINC AND SELENIUM ON RAM SEMEN, INVITRO STUDY
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ABSTRACT
The objective of the current study was to display the effects of addition zinc & selenium on ram semen characteristic in vitro. This study was directed on 3 local breed rams, aged between 2-4 years, presented in the farm of college of veterinary medicine / Al-Fallujah University, at a period of time from the beginning of February to march 2018. The rams were fed with alfalfa and hay. All the experimental animal were treated from internal parasite. semen was collected from each ram by electro-ejaculator apparatus at morning hours at weekly interval. Over a course of 8 week (eight samples per ram) semen sample were put in a water bath with 38°C, pooled semen of both rams were evaluated. The samples of semen were diluted 1: 10 with a tris based extender according to the concentration of spermatozoa (Tris= 24.2 gm, citric acid = 13.4 gm, fructose = 10 gm, glycerin= 64 ml, egg yolk= 192 ml, distal water up to one liter), diluted semen were taken and divided into 3 parts (each part 2 ml), the first part (T1) added to it 4.0 µ selenium / ml of diluted semen. The second part (T2) added to it 0.576µ zinc sulphate / ml of diluted semen, the third part (T3) not added to it anything and serve as a control, another fresh samples were taken and diluted and then cooled gradually via addition a piece of ice till it reaches 4°C within 2 hours, semen parameters were measured after dilution and cooling. The result presented a significant differences at (P<0.05) in an individual motility%, Dead%, alive% & abnormalities% between Zn treated group as comparing with selenium or control group. It was concluded that adding of zinc sulphate to the diluted and cooled semen have a beneficial effect on some ram semen characteristic in non-breeding season.

Introduction
It is believed that an antioxidants are accountable for the objection of sperm against the oxidative stress (Cabrita et al., 2010). It prevents the needless free radicals manufacture (Gutteridge and Halliwell, 1994). The antioxidant are any elements that can prevent the oxidative damaged to the cellular molecules (Aurich et al., 1997). Selenium (Se) is an essential trace elements that maintaining growth and human being as well as animals development. A deficiency of Selinked with particular reproductive problems and reduced the quality of sperm in bull and ram (Chiachun et al., 1991). It has been documented that an adding of Seto the diet can improving the reproductive performance in bull and ram (Baiomy et al., 2009). Selenium functions as a critical antioxidant in animals reproduction (Leonard, 2000; Surai et al., 2000; Barber et al., 2005; Dimitrove et al., 2007). The effects of the antioxidant can be mediated via its functions as a part of the antioxidant enzymes in body particularly the GSH (Eid et al., 2006) and causing constancy of cell membrane. The significant of Se is marked from the previous study facts that the supplementation of Seto the semen shows a preferable storage results along fewer releasing lipid from the sperm throughout storing for a long time (Dimitrove et al., 2007). The provision of Se and / or vit. E can markedly improve the semen characteristic, sexual desire, as well as the plasma testosterone concentrations in goats without changing in the genetic markers (El-sheshtawy et al., 2014).
Supplementations of Se and/or vit.E can improve libido in the buffalo bull (Khalifa, 1997). It has been observed that Se and/or vit.E can enhance the semen characteristics which indicated by an increasing sperm concentration, motility and a live sperm percent and decreased sperm abnormalities in bulls (Rabie, 1992; Ibrahim et al., 1996) & rams (Kendall et al., 2000) and can improve the long life & enhance the quality of cooled semen of rams (Azawi and Hussein, 2013). It has been recorded that addition of selenium and zinc may decrease the influence the heat stress to particular degree at male boars breeding (Horky et al., 2016). There are huge antioxidants from these Se and zinc(Zn) which are export of the antioxidant enzymes particularly the glutathione peroxidase & SOD (Klusionova et al., 2015; Horky, 2015). It has been documented that using of Zn and Se may enhance the status of antioxidative and the hormonal concentrations viarising the Zn & Se levels in the serum as well as seminal plasma (Kumar et al., 2013).

Zinc has a nature of antioxidative and can inhibit the production of ROS and therefore improving the fertility (Bray et al., 1997). Zinc can prevent the lipid peroxidation and settle down the lysosomal membrane (Kimball et al., 1995). The dismutation of the radical superoxide is through the superoxide dismutase enzyme, which contain zinc, its supplementation also can increase catalase in the buffalo seminal plasma (Alavi-shoushtari et al., 2009). Selenium can do as a catalytic center in the vigorous state of various anti-oxidative enzymes (Salvatore et al., 1996) and functions as co-factors of GSHPX system which adjusts both intra cellular and extra cellular peroxidases (Burk and Hill, 1993).

So, Sedeficit can cause aggregation of free radicals that can hurt the cell membrane and disturb the steroidogenesis (Hemler and Lands, 1989), reduce motility of sperm (Alvarez and Story, 1989). Provision of Secan enhancement the semen quality viarising the anti-oxidative protection of sheep seminal plasma (Kendall et al., 2000) and goats (Shi et al., 2010). Zn plays an important role in androgen metabolism, testicular steroidogenesis and steroid receptors interaction (Bedwal and Bahuguna, 1994).

The current research was done to show the effects of Zn and Se on characteristic of ram semen invitro.

**Material and methods**

The current study was done on 3 local breed rams, aged between 2-4 years. Presented in the farm of college of veterinary medicine / Al-Fallujah university. During a period extended from the beginning of February to the march 2018. The animals were feed with alfalfa and hay. All the experimental animal were treat from internal parasite. Semen was collected from each ram by electro-ejaculator (ElectroJac5/ANEOGEN COMPANY) at morning hours. Semen was collected in a graduated tube weekly. Over a course of 8 week (eight samples per ram) semen sample were put in a water bath with 38°C. Pooled semen of both rams were evaluated. 1 ml of semen were taken from each samples used for determination of semen characteristic. Volume of semen was directly measured by reading of graduated marks of collecting tube. The color has been visually measured according to Salisbury et al., (1978). Mass activity by putting a non-cover slipped drop of fresh semen non-diluted semen was placed on a warm slide at 37°C and places under alight microscope with heated stage at 100x magnification. Swirl have been seen in samples with adequate number of motile spermatozoa. The grade of the estimate are according to Chenoweth, (2002). Rapid swirling, very good(VG), slower swirling, good(G), generalized oscillation, fair(F), sporadic oscillation, poor(P). Immediately after collection sample were evaluated for mass motility and individual motility using pre-warmed stage of phase contrast microscope (one drop from fresh semen plus one drop of sodium citrate) and check the percent of individual motility. Semen smears were stained by eosin & nigrosin, were prepared (Blom, 1950), and used to determine the % of live VS dead and morphologically abnormal spermatozoa (primary and secondary) according to Bielanski et al., (1982). Sperm concentration measured by hemocyto to meter chamber (Salisbury et al., 1978).
The samples of semen were diluted 1: 10 with atris based extender according to the concentration of spermatozoa (Tris= 24.2 gm, citric acid = 13.4 gm, fructose = 10 gm, glycerin= 64 ml, egg yolk= 192 ml, distal water up to one liter) according to Eidan (2016) and Aboud (2017). Diluted semen were taken and divided into 3 parts (each part 2 ml), the first part (T1) added to it 4.0 µ selenium /ml of diluent. The second part (T2) added to it 0.576 µ zinc sulphate / ml of diluted semen. The third part (T3) not added to it anything and serve as a control, another fresh samples were taken and diluted and then cooled gradually via addition a piece of ice till it reaches 4c within 2 hours, after cooling the semen it divided into three parts and added to it T1, T2 as in diluted semen. Semen parameters were measured after cooling. Statistical analysis were applied using Tukeys- w procedure and chi- square test according to (steel and torrie, 1980).

Results and discussion

The parameters of fresh ram semen in non-breeding season are shown in (table1), there was no significant difference in semen characteristic between rams and different ejaculates of the same ram. The results showed that the rams of low semen quality, this might be due to non-breeding season collection that affect the fertility of ram (Salisbury et al., 1978).

Table (1) parameters of fresh semen of the ram(Mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume / ml</td>
<td>1.06±0.28</td>
</tr>
<tr>
<td>Color</td>
<td>Creamy</td>
</tr>
<tr>
<td>Concentration (million / ml)× 10^7</td>
<td>145.25±23.54</td>
</tr>
<tr>
<td>Mass activity %</td>
<td>33.8±2.39</td>
</tr>
<tr>
<td>Individual motility %</td>
<td>45.0±2.04</td>
</tr>
<tr>
<td>Dead %</td>
<td>34.3±4.96</td>
</tr>
<tr>
<td>Live %</td>
<td>65.8±4.96</td>
</tr>
<tr>
<td>Abnormalities %</td>
<td>7.50±0.65</td>
</tr>
</tbody>
</table>

Table (2) showed the semen characteristic after dilution with Tris-based extender with addition of zinc sulphate (0.576 µ/ml) and selenium (4 µ/ml) to each of diluted semen. The current results showed a significant difference (P<0.05) in individual motility%, Dead%, alive% and abnormalities% between zinc treated group as compared with selenium or control group.

Table (2) parameters of ram semen after dilution and addition of zinc sulphate and selenium(Mean ± SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual motility %</td>
</tr>
<tr>
<td>T1 / Zinc sulphate (0.576 µ/ml)</td>
<td>42.5±3.23^a</td>
</tr>
<tr>
<td>T2 / Selenium (4 µ/ml)</td>
<td>25.0±2.04^b</td>
</tr>
<tr>
<td>T3 / Control</td>
<td>32.5±1.45^c</td>
</tr>
</tbody>
</table>

Values = Mean ± SE
Different letters showed significant difference (P <0.05)
The beneficial effect of zinc was observed by many investigation in sheep (Shi et al., 1998; Kendall et al., 2000) and in goats (Shi et al., 2010). It has been reported that zinc stimulates the growth and primary & secondary sex organ developments (Kynaston et al., 1988), spermatogenesis (Underwood and Somers, 1969) and especially prostate function (Mann, 1964) in various species.

Zinc has anti-oxidative in nature and can inhibit the production of ROS and therefore improving fertility (Bray et al., 1997). Also it prevents the peroxidation of lipid and stabilize the lysosomal membrane (Kinball et al., 1995). Dismutation of radical superoxide is achieved by superoxide dismutase that contain zinc. It also investigation that zinc supplementation increase catalase in the seminal plasma of buffalo bull (Alavi-Shoushtari et al., 2009).

Table (3) showed the effects of addition Zn and Se after cooling of diluted semen. The present results showed asignificant differences at (P<0.05) between the various treated group in an individual motility %, live %, dead % and abnormalities.

Table (3) parameters of ram semen after cooling and addition of zinc sulphate and selenium(Mean ± SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Individual motility %</th>
<th>Dead %</th>
<th>Live %</th>
<th>Abnormalities %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 / Zinc sulphate</td>
<td></td>
<td>35.0±3.54*</td>
<td>48.5±3.62*</td>
<td>51.5±3.62*</td>
<td>13.8±0.48*</td>
</tr>
<tr>
<td>(0.576 µ/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 / Selenium</td>
<td></td>
<td>8.75±2.50b</td>
<td>73.5±2.90b</td>
<td>26.5±2.90b</td>
<td>25.8±2.06b</td>
</tr>
<tr>
<td>(4 µ/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 / Control</td>
<td></td>
<td>18.8±2.40c</td>
<td>62.3±5.22c</td>
<td>37.8±5.22c</td>
<td>17.8±1.55c</td>
</tr>
</tbody>
</table>

Values = Mean ± SE
Different letters showed significant difference (P <0.05)

The treated group with zinc sulphate showed the best results as compared with selenium and control groups. Similar observation have been made in addition of zinc sulphate to extender of buffalobull semen and give better preservation and higher quality of semen (Dorostkare et al., 2014). It is also known that Zn can prevent DNA & cell membrane of spermatozoa damage and participate in the mechanism of acrosomal reaction and capacitation (Dorostkare et al., 2014).

Conclusions and Recommendation
It was concluded that addition of zinc sulphate to the diluted and cooled semen have a beneficial effect on ram semen characteristic in a non-breeding season so it is recommended to give zinc sulphate in the feed of ram to increased quality of the semen.

References

Azawi, O. I. and Hussein, E. K. (2013). Effect of vitamins C or E supplementation to Tris diluent on the semen quality of Awassi rams preserved at 5 C. In Veterinary Research Forum (Vol. 4, No. 3, p. 157). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.


