Effect of addition of melatonin to the intravaginal sponges on oxidative status and reproductive performance in Iraqi awassi ewes

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Abstract

This study was achieved to know the effect of addition of melatonin to intravaginal sponges impregnated with progesterone on oxidative status and reproductive performance in Iraqi awassi ewes. Twelve non-pregnant ewes were selected with an age ranged between 2-4 years, and weight with a mean of 42.8 ± 5.3 kg. The animals were divided into two equal groups. Each group contained 6 animals. The first group was treated with sponge free from melatonin; the second group treated with sponges contained two grain of melatonin in a dose of 6mg (3mg/grain) by putting them inside the sponges through small opening. All ewes were injected with eCG (250 i.u/ i.m) after the removal of sponges on day 12. Two Rams were introduced on the next day after removal of sponges. Blood samples were collected on day 0, 3, 7 and 12 from insertion of vaginal sponges for measurements the total leukocytes count and the level of malondialdehyde in plasma during the days of treatments with vaginal sponges. The result of study showed a significant increase in the level of malondialdehyde during the days of treatments for both groups. The results also showed that there was no significant difference between two treated groups in percentages of appearance of estrus, the period from removal of sponges to the appearance of estrus, pregnancy rate and fertility rate. It was concluded from this study that the use of vaginal sponges for estrus synchronization in ewes leads to increase the level of oxidative stress and the addition of melatonin with a dose 6mg to the sponges have no effect to decrease the level of oxidative stress that leads to vaginal inflammation concerned the intravaginal sponges.

Key words: Melatonin, Oxidative stress, Reproductive performance, vaginal sponge, Ewes

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Introduction

Intravaginal sponges Impregnated with progesterone considered one of the applicable methods for estrus synchronization in sheep (Manvi, 2014). Usually, vaginal sponges introduced for about 9 to 12 days followed by injection with Equine chorionic gonadotropin (eCG) after removal of sponges from the vagina or before removal with 24 hours (Macías-Cruz et al.,2017). The presence of the sponges in the vagina for this period might be considered as a foreign body that act as a factor predispose to vaginal inflammation(vaginitis), with environmental change of the vagina with aggregation of mucus and pus secretion, that having bad odor due to increase in the total numbers of bacteria. Also there were changes in the commensal and opportunist bacteria in addition to the vaginal fungi that stimulate the occurrence of vaginitis (Suárez et al., 2006 ; Vasconcelos et al., 2016 ; Mohammed et al.,
It is also there was a histological and cytological changes in the wall of the vagina which might be due to the presence of the sponge or contamination resulted from their use (Manes et al., 2015). The changes in the vaginal bacteria and collection of abnormal discharge leads to increase in the percent of non-fertilizable ova in ewes inseminated artificially, and decrease in embryonic development and the next fertility in ewes treated with vaginal sponges (Scudamore, 1988). It has been observed that pregnancy rate decreased significantly in ewes treated with sponges impregnated with progesterone or not as compared with ewes none treated with vaginal sponges (Manes et al., 2014). Manes et al. (2016) found the function and activity of the ram sperm negatively affected when mixed with vaginal mucus taken from ewes treated with vaginal sponges, this might explain the decrease in pregnancy rate in ewes treated with vaginal sponges. Sönmez et al. (2009) found that the use of vaginal sponges in goats leads to increase the level of oxidative stress which might be resulted from vaginal inflammation concerned with the application of sponges. In order to decrease the undesirable effect of vaginal sponges, antibiotics should be added to the sponges before introduced in the vagina. This might leads resistance of bacteria against antibiotics (Martins et al., 2009). In the late few years it becomes more important to use antibacterial factors that are active against resistant bacteria. Melatonin which produced from the pineal gland, have certain functional and multi applicable characteristics such as antioxidant (Tan et al., 1993), antibacterial (Tekbas et al., 2008), anti-inflammatory (Mahmood et al., 2010) and immune stimulator (Halder et al., 2012). Our research theory claimed that the addition of melatonin to the vaginal sponges might reduce vaginal inflammation and the oxidative stress. There is a little information about the relationship between oxidative stress and the use of vaginal sponges. The study was aimed to know the effect of addition of melatonin to vaginal sponges impregnated with progesterone on oxidative status and reproductive performance in Iraqi awassi ewes.

Materials and methods

Experimental animals:
The experimental has been achieved in special farm at hit city, anbar province, Iraq during the period from March 24, 2018 to September 8, 2018. Twelve awassi non pregnant ewes were selected with an age ranged between 2-4 years, with a mean of weight 42.8 ± 5.3 kg. The animals were placed in semi closed system. The animals were fed during the period of the experiment with 2% alfalfa hay from body weight according on dry matter. Also 300g of barley grain per ewe were given. The fresh drinking water and a cubic of mineral licks were available ad libitum.

Experimental design:
The ewes were divided in to two equal groups (6 ewes /group). The first group were treated with intravaginal sponges free from melatonin impregnated with progesterone which contained 60 mg medroxyprogesterone acetate (progespon©, syntax, Argentina), which serve as a control group. The second group were treated with intravaginal sponges contained melatonin in impregnated with progesterone which two grains of melatonin in a dose of 6 mg (3mg /grain) (melatonin, holista health Canada Inc. ), through introduction inside the sponges through small opening. The day of insertion the sponges regarded as on day 0. The vaginal sponges were removed on day 12. All ewes were injected directly after removal of sponges with eCG (NovormonTM 5000, Syntex, Argentina) in a dose of 250 I.U / IM. The rams (2 rams) were introduced in the next day of sponges’ removal. The ewes were observed two times per day for 72 hours after sponges’ removal in order to record the time of estrus appearance. The ewes were inseminated naturally with rams, the pregnancy were diagnosed after 60 days of insemination with ultrasound device (Renco
Pregtone®, USA). Blood was collected (3ml) from the jugular vein via vacutainer tubes containing EDTA at days 0, 3, 7, 12 from the insertion of sponges. The samples were transported to the laboratory with cool box. Total leucocytes count were done (John and Lewis, 1984). Blood plasma was separated with centrifuge with a speed of 3000 rpm/min. for 15 minutes. The plasma put in a small plastic tube and stored at – 20° C till the measurement the level of malondialdehyde (Satho, 1978). The following reproductive indicies has been taken including, The percent of estrus appearance (The numbers of ewes showed estrus/the total numbers of ewes in each group × 100), The period from removal of sponges to the appearance of estrus (hours), Pregnancy rate (The numbers of pregnant ewes / The total numbers of ewes inseminated with rams × 100), Fertility rate (The numbers of ewes showed birth / The numbers of ewes inseminated with ram× 100).

Statistical analysis:
Statistical analyses were done with one way analysis using general linear model with application of SAS program 9.1 (SAS, 2002). Duncan multiple range test were used for the significant difference between means at the level of P< 0.05.

Results
The results of present study showed no losses or expulsion of any sponges till the time of the removal (day 12), for all ewes. All ewes showed a sign of vaginal inflammation after the removal of sponges through the release of mucus and pus discharge with bad odor. Table 1 showed no significant difference in total white blood cells (WBC) count between the first treated group (sponges free from melatonin) and the second treated group (sponges contained melatonin) for the days 0, 3, and 12 from insertion of sponges. While in day 7 there was a significant difference (P<0.0279) in the mean of WBC between the first treatment (11133± 1345 cell/ µl) as compared with the second treatment (7083± 809 cell/ µl). It has been observed that there was no significant difference in the total numbers of WBC at day 0, 3, 7, 12 in the same treatment. Table 2 showed no significant difference in plasma malondialdehyde level between the two treatments for the days 0, 3, 7, and 12 of insertion of sponges. While it showed a significant difference (P<0.0001) in the level of malondialdehyde between days of treatments 0, 3, 7, 12 in the same treatment.

Table 1: White blood cell (cell/ µl) of Iraqi awassi ewes synchronized using intravaginal sponges with or without melatonin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of treatment</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (sponges insertion)</td>
<td>3</td>
</tr>
<tr>
<td>G1 Sponges without melatonin (control)</td>
<td>A 9683 ± 1116’ a</td>
<td>A 9617 ± 940 a</td>
</tr>
<tr>
<td>G2 Sponges with melatonin</td>
<td>A 8800 ± 629 a</td>
<td>A 7467 ± 385 a</td>
</tr>
</tbody>
</table>
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Table 2: Malondialdehyde (µmol/L) of Iraqi awassi ewes synchronized using intravaginal sponges with or without melatonin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of treatment</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days of treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(sponges insertion)</td>
<td>(sponge removal)</td>
</tr>
<tr>
<td>G1 Sponges without melatonin (control)</td>
<td>D 0.038±3 0.010*  a</td>
<td>C 0.0800 ± 0.008  a</td>
</tr>
<tr>
<td>G2 Sponges with melatonin</td>
<td>D 0.0350± 0.012  a</td>
<td>C 0.0616± 0.004  a</td>
</tr>
<tr>
<td>P-Value</td>
<td>n.s**</td>
<td>n.s</td>
</tr>
</tbody>
</table>

* (Mean ± Standard Error)
** n.s: means non-significant.
Means with different letters (a, b) in columns indicate differences among treatments.

Table 3 showed the reproductive performance of Iraqi awassi ewes synchronized with intravaginal sponges free from melatonin or with melatonin. All the ewes of the experiment showed estrus within 45 to 56.5 hours after removal of vaginal sponges. The first treated group showed estrus after 51.3 ± 1.57 hours from removal of sponges. While the second treated group showed estrus after 49.2 ± 2.36 hours from the removal of sponges. There was no significant difference between the two treated groups. The results of pregnancy diagnosis showed 50% pregnancy rate (3/6) in the ewes of the first treated group. While it showed 66.7% (4/6) in the ewes of the second treated group. The fertility percent in the first treated group was 50% while it was 66.7% in the second treated group. Only one ewe showed twin birth in the second treatment.

Table 3: Reproductive performance of Iraqi awassi ewes synchronized using intravaginal sponges with or without melatonin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Number of ewes</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
**Table:**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (6/6)</th>
<th>Group 2 (6/6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of estrus (%)</td>
<td>100</td>
<td>100</td>
<td>n.s.</td>
</tr>
<tr>
<td>Onset of estrus (h)</td>
<td>51.3 ± 1.57</td>
<td>49.4 ± 2.36</td>
<td>*</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>50 (3/6)</td>
<td>66.7 (4/6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fertility rate (%)</td>
<td>50 (3/6)</td>
<td>66.7 (4/6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Number of twining lambs</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Number of newborns</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* (mean±SE )

**Discussion**

Intravaginal sponges impregnated with progesterone have been widely used for induction and synchronization of estrus in sheep. Although the use of intravaginal sponges leads to local inflammation in the vagina companied with collection of pus and bloody discharge with bad odour after removal of sponges. This result was agreed with Martine-Ras et al. (2018), when all ewes (100%) treated with vaginal spong es for 7 days and 94.4% from ewes treated with vaginal sponges for 14 days, showed vaginal discharge after removal of sponges. These discharges were between little transparent to heavy pussy or bloody discharge. The bloody and mucus discharge correlated with histological change includes hyperplasia and hypertrophy of epithelial line of the vagina and cytological changes includes increase in the numbers of epithelial cells, neutrophil, macrophages and red blood cells after the removal of vaginal sponges (Manse et al., 2015). When intravaginal sponges applied, we should added the antibiotics to the sponges before introduction inside the vagina, and this might leads to resistance of bacteria to antibiotics (Martins et al., 2009). So the we turned our attention to added melatonin to the vaginal sponges because having antibacterial activity against the bacteria resist the antibiotics (Tekbas et al., 2008). Also it has been observed in present study that addition of melatonin in a dose of 6 mg not enough to produce effect to prevent or decrease the inflammatory changes a companied introduction of the sponges. Our study explained that the total numbers of WBC not affected significantly during the period of treatment (12 days) for both two groups, and this results agreed with Mansoor (2015) who observed the ear implants of ewes with melatonin have no effect significantly on total numbers of WBC. The application of vaginal sponges leads to increase the level of malondialdehyde which is a good indicator of lipid oxidation during the period of treatment with sponges. The level of malondialdehyde increase gradually till it reaches to its highest level at the day of sponges’ removal for both treatments. This result have been similar to that reported by Sönmez et al.(2000) who found that the level of MDA increased clearly at the days 1-4 after the insertion of the sponges and reached its higher level at the day of sponges removal. The increase level of MDA might be due to occurrence of inflammation accompanied the use of vaginal sponges.

In our present study it was not observed any effect to the addition of melatonin to vaginal sponges on reduction of oxidative stress resulted from the use of vaginal sponges. These results were disagreed with Choudhary et al. (2018), who found that when melatonin given orally or subcutaneously leads to decrease in the level of MDA and increased significantly in the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes. The melatonin act directly

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for removal of free radicals in different types such as reactive oxygen species, in addition to active types of nitrogen reactive species. This might reduce the oxidative damage in the cells (Maharaj, 2003). Also acts indirectly as antioxidant through activation of antioxidant enzyme mRNA and finally production of main antioxidant enzymes such as superoxide dismutase, glutathione peroxidase (GPx) and catalase enzymes (Rodriguez et al., 2004). The cause of no effect of melatonin added to sponges might be due to the dose of melatonin 6 mg is not enough.

It has been reported the treatments with progesterone and eCG one of previous methods for induction and synchronization of estrus out of breeding season in ewes. Our study showed 100% of estrus appearance in both treatments. While the fertility rate was 58.3 % (7/12). These results were lower than reported in other studies when the fertility rate ranged between 60 – 97 % (Manes et al., 2010; Martinez-Ros, 2018; Yilmezer et al., 2018).

The lower results in pregnancy rate in our study might be due to the difference in breed, environment or the effect of vaginal inflammation that accompanied with oxidative stress during treatment with vaginal sponges.

It was concluded from this study that the use of vaginal sponges for induction and synchronization of estrus in sheep might leads to oxidative stress and addition of melatonin in dose 6 mg to the sponges have no effect to reduce oxidative stress accompanied with vaginal inflammation. However more research is recommended.

**Reference**


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