Is there a relationship between serum minerals (Ca, Mg) and trace elements (Cu, Fe, Mn, Zn) at mating on pregnancy rates in fat-tailed Morkaraman sheep?*

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ABSTRACT

The specific roles of nutrients in reproduction are not well defined in sheep. Hence, the relationships between certain mineral (Ca, Mg) and element (Cu, Fe, Mn, Zn) levels at mating and pregnancy rates was investigated in fat-tailed Morkaraman ewes synchronised with different hormones (melatonin, norgestomet implant and eCG) early in anoestrus season. For this purpose, 40 healthy ewes (aged 2-4 years) allocated in the synchronised (n=30) and no-treated group (n=10) were used during mid-May (suckling period). On the eCG injection day, fertile rams (n=8) were introduced into the groups and, then the oestrus signs were visually monitored twice daily for 7 days. The oestrus ewes were allowed to hand-mate using rotational (four rams daily) mating system. Additionally, jugular blood samples were collected on the days of onset of oestrus (before mating) for determination of serum Ca, Cu, Fe, Mg, Mn and Zn levels. Presence of pregnancies was diagnosed by real-time ultrasonography 35 days post-mating. Totally, 25 ewes became pregnant (21 ewes with stimulated- and 4 with spontaneous oestrus), while 9 ewes (all received stimulation) were not pregnant. In non-pregnant ewes, unlike other minerals and elements, the Cu and Zn values were significantly lower than their reference values (for Cu: 0.87±0.16 mg/dl vs. 9.20 mg/dl; for Zn: 2.06±0.30 μg/dl vs. 3.8 μg/dl, resp.; P<0.01 in both cases). In pregnant ewes, the corresponding values remained unchanged (P>0.05). Hence, the present findings indicate that the low Cu and Zn levels during the oestrus might adversely affect the subsequent pregnancy rate in suckling ewes.

Key words: Anoestrus, Ewe, Elements, Pregnancy, Serum minerals, Synchronisation.

INTRODUCTION

In farm animals, healthy parturition (herein lambing) is the final outcome of reproduction. So, the main objective of mating in sheep flocks, as in other farm animals, is to maximize the number of lambs born (litter size). For a superior fertility, non-pregnant animals should be kept within the flock for a long time during the breeding season. However, if the presence of these animals unable to become pregnant due to unknown reasons continues in a flock, that will be economically risky for animal enterprises.

To reach the optimum pregnancy rate in a given female population, many factors that would prevent pregnancy collectively could be possible only when we know the actual reasons very well (Alaçam et al. 1997). These factors could be attributed to the environmental and/or maternal reasons. However, there exists no adequate research about the infertile animals showing healthy oestrus in breeding season but not becoming pregnant even though being mated. For example, some trace elements (Zinc-Zn, Manganese-Mn, Copper-Cu, Selenium-Se, Iodine-I) could affect fertility as being a structural element of many enzymes and hormones. For example, many reproductive hormones are directly related with the Zinc (Yaylali and Sözer 1995). These hormones require the Zn in order to show their effects

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at the receptor points in target tissues (ovary, uterus). Again, the zinc has a critical role to play in the differentiation of embryonic cell division and fertilisation (Aksakal et al., 1991; Sandabe et al., 2004; Gürdogan et al., 2006; Yokus et al., 2006, Uslu et al., 2012a). Based on these characteristics, the blood levels of mineral and trace elements needed especially in reproductive season draws our attention due mainly to their effects on pregnancy (Mc Dowell 1992).

Therefore, the aim of this study was to investigate the effects some minerals and trace elements on pregnancy rates in fat-tailed Morkaraman sheep in breeding season. The present study to be conducted in clinically healthy flocks but with low pregnancy rates would contribute towards developing proper nutrition strategies in sheep breeding.

MATERIALS AND METHODS

Location and season: The experiment was conducted from June to July at the Research Farm Station, Yuzuncu Yil University, Van (38°29'39"N and 43°22'48"E), Turkey. The region has an altitude of 1,727 m and is characterised by an average annual temperature of 13-17 °C. The study was conducted from May to July, with 13.5 h-14.5 h daylight.

The experiments were performed in early anoestrus (suckling) season under a natural photoperiodic environment.

Animals: Ewes were maintained outdoors from May to November. After grazing, they were housed in pens and fed with grain oats and hay diets. Water and mineral licks were offered ad libitum. Thirty-four mature (2–4 years), non-lactating ewes weighting (body weight, b.w.) from 45 to 56 kg (49.9±1.2 kg, mean ± SD) were used as material. Body condition scores, BCS (1-5 scale; 1-emaciated to 5-obese) ranged from 3.0 to 4.0 with an average of 3.5±0.4 (Gibon et al., 1985). Differences neither in the average b.w. nor in the BCS’s between the groups were significant.

Experimental groups: The animals were randomly divided into two groups, as follows:

i) Control (CON) group (n=10); ewes, as control, control received no treatment.

ii) Synchronisation (SYNCH) group (n=30); ewes received various hormones. They were synchronised by using melatonin (MEL), norgestomet (NOR), and melatonin + norgestomet (MELNOR) as well as eCG (for all treatment groups). Details of these protocols and their preliminary results have already been published elsewhere (Uslu et al., 2012b).

On the day of eCG injection, fertility-proven rams were introduced to the flock and all the ewes were then all monitored twice daily (between 07:00 a.m.-18:00 p.m.). Once the ewes were detected in oestrus, they were allowed to hand-mate. The oestrus response and pregnancy rate were recorded.

Blood collection: Samples of blood were collected by jugular venipuncture on:

1. the day of hormone administrations, ii) the day of their withdrawals (Day zero), and iii) the day of oestrus. Blood parameters were analysed in the Laboratory of Physiology Department in Faculty of Veterinary Medicine at Yuzuncu Yil University. On the days specified, both 5 ml (into vacuum tubes, with EDTA) and 10 ml (into the tubes, without EDTA) of samples were taken on Days -35, -10, zero and Oestrus (Table1).

Samples were separated (as serum and plasma) by centrifugation at 3,000 rpm for 15 min and stored at -20 °C until the analyses.

Blood analyses: Measurements of serum mineral values were calculated by atomic absorption spectrophotometry and autoanalyser methods, given elsewhere (Uçar et al. 2011; Uslu et al., 2012a). Atomic absorption spectrophotometry equipped with a computer (Unicam 929-UK) was used for biochemical analyses of Mg (mg/dl), Cu (mg/dl) and Ca minerals (mg/dl). Also, the analyses of Zn (µg/dl), Fe (µg/dl) and Mn (µg/dl) were performed by autoanalyser (TECNICON®, Seal Analytical, Wisconsin, USA) according to their specific methods, directed by the manufacturer guidelines (Morton and Robert 1993; Longbottom et al., 1994).

Statistical analyses: Statistical differences of mineral and trace element levels between synchronisation groups were determined by the Student’s t-test and Duncan’s repeated measures test. However, data (for example, the numbers of oestrous- and lambing ewes) were analysed by the x² test using the SPSS 12.0 software (SPSS 2013). A difference was considered significant when P<0.05.

RESULTS AND DISCUSSION

Oestrus and pregnancy rates of ewes according to synchronisation protocols are given in Table 1. The levels of Mo, Zn, Mg, Cu, Ca and Fe in blood sera of control and treated ewes were determined on: i) the initial day of study (on Day -35, for all groups except the NOR group); ii) Day -10, iii) the day of removal of implants (Day zero) and iv) oestrus days (Oestrus Day) and are given in Table 2. In the CON, MEL, and MEL+NOR groups, samples were taken on the day -35, while in the NOR group, the first samples were taken on the day -10.

Additionally, for minerals and trace elements studied, the reference values (Table 3), as well as the values on:

1. the beginning day (Table 5), ii) the last day of hormonal administration (Table 4) iii) the day of oestrus (Table 4) iv)

<table>
<thead>
<tr>
<th>Table 1: Days of blood collection (BC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CON</td>
</tr>
<tr>
<td>MEL</td>
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<tr>
<td>NOR</td>
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<tr>
<td>MEL+NOR</td>
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</tbody>
</table>
the 75th day of pregnancy (Table 4) in control and synchronised ewes are also given herewith.

Creating a number of enzymes and hormones essential elements of trace elements, it is known to affect fertility. However, the data indicating the physiological limits of the sheep in the blood parameters are quite limited in both literature textbooks. These values are remarkable in that they differ from each other. In this study, the first place in the pre-vaccine reduced blood Cu and Zn values were detected in the negative on pregnancy. Our study, especially fed on natural pasture conditions and pregnant animals are more that did not put a lot in, excess blood values checked at the entrance to the season in terms of Cu and Zn reveals the necessity.

Herein, we investigated the relationship between the levels of Ca and Mg minerals as well as Cu, Fe, Mn and Zn elements at mating and subsequent pregnancy rates in fat-tailed Morkaraman ewes synchronised with various hormonal treatments (melatonin, norgestomet implant and eCG) in the early anoestrous season, as details given elsewhere (Uslu et al., 2012b). Although the low serum Cu and Zn levels during the oestrus adversely affected the subsequent pregnancy rate, but no marked change in mineral levels was found between different synchronisation protocols in suckling (anoestrous) season. Meanwhile, the course of Ca, Cu and Zn levels were also remained low in the control ewes receiving no treatment.

Within the organisms, the large part (99%) of Ca (mg/dl) is found in combination with Phosphorus (P) in bones. It is reported that many hormones (such as oestrogens, corticosteroids, growth hormone, glucagon, and thyroxine) influence the Ca absorption (Turgut, 2000). In addition, the control of Ca levels in blood is also affected by many factors, of which the hormonal changes in pregnancy is reported to be the most effective (Capes and Rosolini, 1989). Likewise, Antunovic et al. (2002) indicated that Ca increased in the final stages of pregnancy, while Roubies et al. (2006) noted that it increased after the birth. Dutta et al. (1988) stated that serum Ca in the cyclic animals did not differ between the groups. Rao et al. (1981) reported that although the Ca level of cows having a normal oestrous cycle was not

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Table 2: Oestrus and pregnancy rates of Morkaraman ewes (n=40) synchronised by various methods given (Uslu et al., 2012b)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reproductive parameters</th>
<th>Oestrus Response</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group CON (n=10)</td>
<td></td>
<td>40±16.3a</td>
<td>50±28.9a</td>
</tr>
<tr>
<td>Group MEL (n=10)</td>
<td></td>
<td>100b</td>
<td>70±15.3b</td>
</tr>
<tr>
<td>Group NOR (n=10)</td>
<td></td>
<td>100b</td>
<td>60±16.3b</td>
</tr>
<tr>
<td>Group MEL+NOR (n=10)</td>
<td></td>
<td>100b</td>
<td>80±13.3b</td>
</tr>
</tbody>
</table>

a-b: Means having different superscripts within the same column are significantly different from each other (P<0.05).

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Table 3: Mineral levels of ewes in Group CON (n=10) on different days of synchronisation

<table>
<thead>
<tr>
<th>Days post synchronization</th>
<th>Ca (mg/dl)</th>
<th>Cu (mg/dl)</th>
<th>Fe (µg/dl)</th>
<th>Mg (mg/dl)</th>
<th>Mn (µg/dl)</th>
<th>Zn (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/dl)(X ± Sx)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestrus Day</td>
<td>110.4±13.67a</td>
<td>0.83±0.15</td>
<td>1.93±0.73</td>
<td>3.34±0.63</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Group CON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day -35</td>
<td>94.39±24.6</td>
<td>1.05±0.5</td>
<td>2.19±0.46</td>
<td>3.38±0.78</td>
<td>0.05±0.01</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Group MEL+NOR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day -35</td>
<td>103.24±16.41</td>
<td>0.83±0.15</td>
<td>1.93±0.73</td>
<td>3.34±0.63</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
</tr>
</tbody>
</table>

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*Means having different superscripts on the same line are significantly different from each other (P<0.05).*
different from those in the repeat breeders, while Kumar et al. (1986) noted that the levels of cyclic cows were higher than those in anoestrus and infertile cows. Borella et al. (1990) argued that an increased Ca/Mg ratio led to the pregnancy pathologies and spontaneous abortions. For this reason, Mg supplement has to be included in rations to avoid pathologies. At the beginning of this study, there was no statistically significant difference between the SYNCH and CON groups (P>0.05). Following the hormone administrations for synchronisation, only a slight increase in serum Ca levels was observed in the SYNCH group, while there was a marked reduction in the CON group (P<0.05). On the day of oestrus, the levels in SYNCH group returned to previous values, but with a marked increase in the CON group (P<0.05). But, in the subsequent period, on the 75th day of pregnancy, the Ca values approached down to the baseline levels in the CON group, while those in the SYNCH group decreased below the baseline levels (P<0.05). Differences observed in both groups might simply be due to the obvious effect of on going lactation and/or pregnancy. Indeed, these physiologically very demanding statues both require different levels of Ca usage (mobilisation). Undoubtedly, we can expect that in lactating (suckling) pregnant ewes (in SYNCH group), elevated concentrations of Ca are to be released from the endogenous resources (bones in particular) and be used for sustainable pregnancy.

The physiological condition affects the absorption of Cu, and this absorption is increased in pregnant and newborn individuals (Günay, 1996). As of great importance in animal feed, the levels of serum Cu (mg/dl) in sheep are reported as average from 0.80 to 1.20 ppm (Lorenz and Gibb, 1975). In Turkey, its level in clinically healthy Morkaraman sheep has been reported to be 0.75 ppm (Kumar et al., 1986). In our study, the serum Cu concentrations given remained at lower than the reference values (due likely to the effect of lactation and/or pregnancy) and unchanged (P>0.05) between different synchronisation programmes and this was in line with the data concerned.

When serum levels of the Fe (µg/dl) is examined in accordance with groups, the most marked (P<0.001) change was observed in Group NOR. Iron is a mineral acting as 1.66-2.22 µg/dl in many areas of metabolism (Phillippo et al., 1987). However, Antunovic et al. (2002) reported that

<p>| Table 4: Reference values of various minerals and trace elements in ewes (Fraser, 1991; Turgut 2000). |</p>
<table>
<thead>
<tr>
<th>Minerals</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/dl)</td>
<td>63.5-160</td>
</tr>
<tr>
<td>Cu (mg/dl)</td>
<td>9.20</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>2</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>2.2-2.8</td>
</tr>
<tr>
<td>Mn (µg/dl)</td>
<td>0.27</td>
</tr>
<tr>
<td>Zn (µg/dl)</td>
<td>3.8</td>
</tr>
</tbody>
</table>

<p>| Table 5: The values of minerals and trace elements in control (n=10) and synchronised ewes (n=30) on the treatme nts days |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Ca (mg/dl) (X ± Sx)</th>
<th>Cu (mg/dl) (X ± Sx)</th>
<th>Fe (µg/dl) (X ± Sx)</th>
<th>Mg (mg/dl) (X ± Sx)</th>
<th>Mn (µg/dl) (X ± Sx)</th>
<th>Zn (µg/dl) (X ± Sx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Day</td>
<td>CON</td>
<td>104.01±11.67</td>
<td>2.24±0.09</td>
<td>2.38±1.54</td>
<td>1.66±0.34</td>
<td>1.08±0.23</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>SYNCH</td>
<td>103.38±15.00</td>
<td>2.16±0.20</td>
<td>2.50±0.75</td>
<td>1.82±0.46</td>
<td>0.78±0.09</td>
<td>0.37±0.07</td>
<td></td>
</tr>
<tr>
<td>On the last day of hormone administration</td>
<td>CON</td>
<td>105.38±15.67</td>
<td>2.16±0.20</td>
<td>2.50±0.75</td>
<td>1.82±0.46</td>
<td>0.78±0.09</td>
<td>0.37±0.07</td>
</tr>
<tr>
<td>SYNCH</td>
<td>105.5±14.29</td>
<td>2.16±0.20</td>
<td>2.50±0.75</td>
<td>1.82±0.46</td>
<td>0.78±0.09</td>
<td>0.37±0.07</td>
<td></td>
</tr>
<tr>
<td>On the 30th day of pregnancy</td>
<td>CON</td>
<td>75.10±19.12</td>
<td>2.60±1.15</td>
<td>2.25±0.21</td>
<td>1.24±0.55</td>
<td>0.26±0.10</td>
<td>2.50±1.25</td>
</tr>
<tr>
<td>SYNCH</td>
<td>46.21±9.92</td>
<td>2.23±1.87</td>
<td>2.88±1.66</td>
<td>3.22±2.17</td>
<td>0.08±0.49</td>
<td>2.90±1.28</td>
<td></td>
</tr>
</tbody>
</table>

a-b: Means having different superscripts within the same line are significantly different from each other (P<0.05). CON: Control, SYNCH: Synchronised.
although there was a marked increase in serum Fe levels depending on foetal growth particularly during the last trimester of pregnancy, while the levels decreased immediately before the lambing of Akkaraman ewes. This decline is thought to occur due to the usage of Fe (Gürdogan et al., 2006).

Mg is important particularly for ruminants (Ammerman and Goodrich, 1983). Hypomagnesemia is often occurs along with hypocalcaemia (Kalaycioglu et al., 1998). Yokus et al. (2004) reported that the concentration (mg/dl) of Mg was unaffected by physiological changes in Sakis-Awassi sheep. In the present study, there was a marked (P<0.01) decrease of Mg level only in the MEL group of the synchronisation protocols in the oestrus day. A similar (P<0.01) decrease in sheep showing oestrus was also observed in the CON group. The resulting parameters are in line with the literature (Yokus et al., 2004).

Manganese (Mn) is a trace mineral required for protein metabolism and lactation. It is a component of some enzymes and stimulates the development and activity of other enzymes. Mn deficiency in cows resulted in lower conception rate, silent heat and abortion (Hansen and Spears 2008). Male reproduction is also affected by low Mn status that inhibits libido and lowers the numbers of spermatozoa (Chaudhary et al., 2003). Another consideration for bull fertility is bone soundness and travelling ability. Feeding beef cattle with ration involving complex Cobalt (Co), Zinc (Zn) and Mn has been shown to enhance reproductive performance early in breeding season. Inseminating cows earlier upon calving could facilitate to limit the breeding period and allow for obtaining a heavier calf in the following year at weaning (Kirchhoff, 2012). Inseminating females given complex minerals in rations could yield to a 35% improvement in conception rates following the AI as compared to those cows given inorganic forms of minerals (Anonymous 2). In the present study, serum Mn level in all groups was 0.03±0.01 μg/dl on the -35th day, while the highest increase (P<0.001) was reached to 2.18±0.42 μg/dl in the NOR group on Day zero. A considerable increase of 0.12±0.08 μg/dl was also found in the MEL group. Meanwhile, in the CON group, there was a marked (P<0.05) increase from 0.063±0.02 μg/dl on day -10 to Day zero, respectively. In this respect, it is reported that the low circulating levels of Mn in cattle lead to silent heat, lower pregnancy rates and abortion (Ahola et al., 2004). In our study, however, no marked reduction in Mn levels was observed in any group.

Zn is an essential element for normal growth, development and reproduction of animals (Perry, 1990). In particular, it is essential for the development and spermatogenesis of the secondary sex organs in men (Cheah and Yang, 2011). Çimtay and Ölçücü (2000) reported in females that, during different stages of all reproductive events (from the oestrus to the birth and lactation) it plays an active role such that in Zn deficiency oestrus irregularities, infertility, decreased birth weight and stillbirth occur. Levels of mean serum Zn (μg/dl) have been reported as average of 0.40 ppm for Morkaraman breed (Kaya et al., 1998). Bediz et al., (2002) found that the plasma zinc levels in the melatonin group were markedly higher than in the other groups studied. Additionally, the pineal gland and the antioxidant effect of melatonin as well as its relationship with the immune functions have also been the subject of numerous studies (Maestrioni 1993; Üstündag and Canatan, 1999). The effects of melatonin on the immune system have been reported to occur via zinc. Melatonin, the main neurohormone synthesised and secreted by the pineal gland, has been suggested to increase the absorption of zinc within the digestive system. In the presence of specific binding sites for melatonin in the intestine, it is seen as an essential mechanism for increasing the absorption of zinc in the digestive tract (Mocchegiani et al., 1994). In our study, the presences of: i) a marked increase (P<0.001) of Zn in the MEL group on Day zero as compared to Day -35, ii) a further increase (P<0.01) in the MELNOR group on Day -10 as compared to the Day -35, and iii) its continuation during the oestrus are all in line with the literature (Mocchegiani et al., 1994; 1996).

On the other hand, it is a period that prepartum hormonal and strong oxidative stress. However, pre-insemination, especially oxidative stress and the effects of fertilization and pregnancy rates is unknown. In future should work that occur during oxidative stress and oestrus, especially the effects on blood Cu and Zn values and implications of these effects on the rate of pregnancy it should be investigated.

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