ABSTRACT

A study was conducted on 30 adult Iraqi ewes and 5 fertile rams. Ewes were divided into 3 groups 10 animals each, first group treated with melatonin implants (18 mg / animal) for 35 days, second group was treated with intra-vaginal sponges for 12 days followed by injection of PMSG (600 IU / IM) at the time of sponges withdrawal, third group was injected with distilled water (1 ml / IM) and considered as control group. Blood samples were collected from all ewes for estimation of progesterone concentrations at 4 hours before treatments, 10 days after treatments, at 0, 10, 25, 40, 55, 70 and 85 days of pregnancy. Serum progesterone concentrations in group two at estrous phase, 10, 25, 40, 55, 70 and 85 days of pregnancy were (0.30, 6.90, 8.90, 9.10, 10.20, 9.30 and 11.30) respectively showed increasing significantly with group one (0.20, 5.80, 6.57, 7.90, 8.80, 7.80 and 8.20) and group three (0.10, 4.20, 5.20, 6.20, 7.50, 5.90 and 7.10) respectively at (P≤0.05), while group one increased significantly at (P≤0.05) compared to group three. In conclusion the administration of progesterone to ewes by intra-vaginal sponges elevates the P4 concentrations significantly as compared to melatonin and control groups.

Key word: Progesterone, melatonin, lactating ewes, PMSG.

Introduction

Ewes are seasonally polyestrous animals. Its sexual activity begins in autumn, when daytime declines and the heat falls (Pineda and Dooley, 2003; Kirkwood et al., 2012). To determine the reproductive success of production animals, the hormonal evaluation tools are essential. In ewes, Progesterone is a corpus luteum hormone (a yellow body in the mammalian ovary, where follicular cells grow into corpus luteum) that is synthesized in order to prolife the endometrium in an embryo preparation (Field and Taylor, 2012). It is necessary in all mammals for pregnancy establishment and maintenance. PG can be used for implantation and positioning during early embryonic development, and if the levels of PG are inadequate, pregnancy can fail and therefore early embryonic loss (Fernandez et al., 2018) with unsuccessful embryos, which are normally lost in maternal recognition before a 13-day critical stage, does not affect the duration of the ewe cycle. The fetal cycle starts after 35 days of gestation and lasts between 145-150 days until the entire gestation is complete (Ortega-Mora, 2007). Sheep PG hormone therapies may be employed in anoestrian ewes to cause cycling. These treatments have proven to increase pregnancy and conception rates and to enable producers to synchronize oestrus with sheep to make reproductive management easier (Knights et al., 2011). The ewe’s reproductive system is particularly stress-prone. This is due to the effects of stress on reducing GnRH and LH development that can obstruct or delay the growth of follicles in the breed that lead to subfertility(Joseph and Whirledge, 2017). The aim of this study was to investigate the efficiency of intra-vaginal sponges of progesterone and PMSG with subcutaneous implants of melatonin administration and its effect on progesterone concentrations from treatments until 90 days of pregnancy.

Materials and methods

This study was carried out at a local field of sheep in Iraq. Thirty local breed ewes were aged (3-5 years) with an average body weight of (40-50 kg) with a history of one lambing at least and five fertile rams were aged (4-6 years) were used for detecting estrus and service. Before starting the
experiment all ewes were submitted to trans-rectal and trans-abdominal ultrasonography and vaginal inspection by vaginal speculum to be sure that ewes were non pregnant and free from any infection or abnormalities. All ewes were signed by different numbers and colors, then the females were isolated from the males to avoid sight, smell and hearing. The ewes were divided to three groups randomly, group 1 was treated with melatonin hormone, group 2 was treated with vaginal sponges and group 3 was the control group.

Group 1: Ten ewes were treated with ear implant melatonin hormone containing 18 mg (Melovine®, Ceva Santé Animale, France) for 35 days. All ewes were serviced naturally twice a day with fertile rams at standing heat, mating day was considered as day 0 for gestational period calculation. Group 2: Ten ewes were treated by insertion of intra-vaginal sponges having 60 mg Medroxy progesterone acetate (ESPONJAVAVER®, Girona - Spain) twelve days and injection of PMSG (600 IU / IM, ÇÖZÜÇÜ, Girona, Spain) at the time of sponges removal. All ewes were serviced naturally two times per day with fertile rams at standing heat. The mating was began at 32h after sponge removal and injection of eCG, mating day was considered as Day 0 for gestational period calculation. Group 3: Ten ewes injected with distilled water (1ml / IM) and considered as control group. The blood samples were collected for all groups before treatments, after treatments, at estrous phase and every 15 days until 85 days of pregnancy. Then 5 ml of blood is drained into vacuum tubes from the jugular vein (Gel and Clot Activator), these have been put in a cool box till centrifugation. Serum was separated by centrifugation after collection at 3000 RPM for 20 min, and then stored at -18 ° C before progesterone concentrations are assayed. P4 concentrations measured via ELISA Test System. The kit was provided by Monobind Inc. Certified Company, Lake Forest, USA. P4 Review was carried out on the basis of the company's measures, p4 concentration (ng/ml) was measured using Micro plate Enzyme Immunoassay, Colorimetric (Automatic ELISA Reader, Italy). The analytical sensitivity of the assay was 0.105 ng /ml, whereas the specificity was 100% for progesterone.

Statistical Analysis
The software Statistical Analysis Method- SAS (2012) was used.

Results
The current study result illustrated that serum P4 concentration at 4 hours before treatment in group one (0.98) was significantly (P≤0.05) increased compared to group three (0.89), while there were no significant differences compared to group two (0.92). At 10 days after treatment, group two (2.10) showed significant difference compared to group one (0.76) and group three (0.82) at (P≤0.05). The concentrations of P4 in group two at estrous phase, 10, 25, 40, 55, 70 and 85 days of pregnancy were (0.30, 6.90, 8.90, 10.20, 9.30 and 11.30), respectively showed increasing significantly (P≤0.05) with group one (0.20, 5.80, 6.57, 7.90, 8.80, 7.80 and 8.20) and group three (0.10, 4.20, 5.20, 6.20, 7.50, 5.90 and 7.10), respectively, while group one increased significantly (P≤0.05) compared to group three as shown in Table 1.

<table>
<thead>
<tr>
<th>Time of collection</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of P4 ng/ml ± SE</td>
<td>Mean of P4 ng/ml ± SE</td>
<td>Mean of P4 ng/ml ± SE</td>
<td></td>
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</tr>
<tr>
<td>4 hrs before treatments</td>
<td>0.98 ± 0.02 a</td>
<td>0.92 ± 0.02 ab</td>
<td>0.89 ± 0.05 b</td>
<td>0.078 *</td>
</tr>
<tr>
<td>10 days after treatments</td>
<td>0.76 ± 0.03 b</td>
<td>2.10 ± 0.12 a</td>
<td>0.82 ± 0.04 b</td>
<td>0.461 *</td>
</tr>
<tr>
<td>At estrous phase</td>
<td>0.20 ± 0.02 b</td>
<td>0.30 ± 0.03 a</td>
<td>0.10 ± 0.01 c</td>
<td>0.0688 *</td>
</tr>
<tr>
<td>10 days of Pregnancy</td>
<td>5.80 ± 0.36 b</td>
<td>6.90 ± 0.24 a</td>
<td>4.20 ± 0.03 c</td>
<td>0.933 *</td>
</tr>
<tr>
<td>25 days of pregnancy</td>
<td>6.57 ± 0.12 b</td>
<td>8.90 ± 0.28 a</td>
<td>5.20 ± 0.14 c</td>
<td>1.074 *</td>
</tr>
<tr>
<td>Days of Pregnancy</td>
<td>Mean Progesterone Concentration (ng/ml)</td>
<td>SEM</td>
<td>p-value</td>
<td></td>
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<tr>
<td>-------------------</td>
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<tr>
<td>40 days of pregnancy</td>
<td>7.90 ± 0.33 b</td>
<td>9.10 ± 0.16 a</td>
<td>6.20 ± 0.11 c</td>
<td>1.392 *</td>
</tr>
<tr>
<td>55 days of pregnancy</td>
<td>8.80 ± 0.26 b</td>
<td>10.20 ± 0.16</td>
<td>7.50 ± 0.23 c</td>
<td>1.286 *</td>
</tr>
<tr>
<td>70 days of pregnancy</td>
<td>7.80 ± 0.27 b</td>
<td>9.30 ± 0.24 a</td>
<td>5.90 ± 0.26 c</td>
<td>1.207 *</td>
</tr>
<tr>
<td>85 days of pregnancy</td>
<td>8.20 ± 0.12 b</td>
<td>11.30 ± 0.16 a</td>
<td>7.10 ± 0.06 c</td>
<td>0.995 *</td>
</tr>
</tbody>
</table>

Means with different letters in the row are significantly different. * (P≤0.05).

Discussion

In the present study, the serum progesterone concentrations (ng/ml) of group 2 (vaginal sponges + PMSG) at 10 days after treatment was 2.10 ± 0.12. However, the serum progesterone concentrations (ng/ml) of group 1 and group 3 were 0.76 ± 0.03 and 0.82 ± 0.04, respectively (Table 1). These results were disagreed with D’Souza (2013), who reported that the progesterone concentration at the time of CIDR insert was 0.6 ng/ml, and at the time of the removal of the CIDR was 0.4 ng/ml, Ainsworth and Downey (1986) found that the serum progesterone concentrations was 1.7 ng/ml at the day of CIDR removal. Such differences may be due to the difference dose of MAP or age of ewes.

In group 2 (vaginal sponges + PMSG) the serum progesterone concentrations (ng/ml) on day 0 estrus was 0.30 ± 0.03. These results were in agreement with Swelum et al. (2015), they reported that the levels progesterone concentrations were 0.2 to 0.4 ng/ml at the CIDR withdrawal and after 24 hours. Also; Beard et al. (1991), also found that the level of progesterone concentration at estrus was 0.5 ng/ml. However, this result was lower than that reported by D’Souza (2013), were the levels of plasma progesterone concentration between 3rd and 4th days of the treatment end were 2.8 and 3.4 ng/ml respectively, when synchronized ewes with CIDR and CIDR + FSH, respectively. Goodman (1994) stated that progesterone concentration decreases at the end of the cycle and then tonic LH levels rise to reach values at least five fold greater than the base line by the time of the onset of the pre ovulatory LH surge, this increase triggers events in those follicles destined for ovulation at estrus.

In this study, there was an increase in the progesterone concentration during gestation period from the first 25 day until the end of 85 days gestation in all groups. Similar result was found by Hussain (1987) and Alwan et al. (2010), that the progesterone concentrations during gestation period were 6.70±1.01, 7.75±0.75 and 15.71±1.53 ng/ml, at the (first, second and third) months of gestation respectively. Similarly, Ranilla et al. (1994) reported that the progesterone concentrations during gestation period were 5.0 to 6.0, 8.0 to 10.0 and 10.0 to 12.0 (ng/ml) at first, second and third months respectively after synchronized ewes by 60 mg MAP for 14 days and injection with 300 IU PMSG at the removal sponges. This strongly affirmed the role of progesterone in enhancing the embryo survival which leads to good conception values in such groups, this in accordance with Mulvaney (2011). Increasing of progesterone level during early pregnancy reduce embryonic losses and increase pregnancy rate and fertility (Ataman et al., 2013).

However this result was lower than that recorded by Youtov (2007), they reported that the serum progesterone concentration were 11.1±3.8, 15.2±4.1 and 20.1±3.0 (ng/ml) after 20, 40 and 60 days of insemination respectively when synchronized ewes by intra-vaginal sponge. In addition, John William (1992) reported that the progesterone levels on days 73, 94 and 116 of gestation period were 5.6, 11.1 and 18.6 (ng/ml) respectively when treated ewes with PGF2α. Furthermore, Abu-Ghazal (2010) found that the progesterone concentration after 15 days from sponges removal was 2.7 ng/ml when synchronized ewes by 60 mg MAP and injection with 300 IU PMSG at the sponge removal. Weems et al. (2007) stated that the CL must be maintained and continue to produce high concentrations of progesterone at least until the developing placenta can assume responsibility for progesterone production. In sheep this transition from CL dependent to placenta-dependent progesterone production occurs as early as 55-90 days after conception (JC et al., 1991).
Yilmazer et al. (2018) whom recorded that progesterone concentration at melatonin treated group were 0.36±0.03, 1.42±0.34 and 4.46±0.48 at time of melatonin implant, 7 days after treatment and 20 days after rams introduced, respectively, this results were in accordance with current study results (table 1.), these variation in values may be due to breed of ewe, physiological status of ewe, geographical location and sampling time.

References


