Introduction

Livestock industry especially in Iraq and other growing countries suffer from a serious deficit to provide the raising demand for animal products (1). In this scope, improving reproductive performance could help to increase animal production. Progesterone is a steroid hormone, have an important functions in reproductive sexual behavior, preparation of the uterus and implantation of the embryo (2,3). The hormone is synthesized mainly in the ovaries, placenta and also secreted from adrenal Cortex as well as from Central nervous system (CNs) in both male and female animals (4,5). Several species females had a higher progesterone levels a plasma than males (6). Treatment of ovarian dysfunction induced

This study was conducted to determine the effect of bone marrow stromal cells (BMSCs) on progesterone level during pregnancy in ewes. Flurogestone Acetate Sponges 40 mg, followed by 400 i.u. Equine chorionic gonadotropin (eCG) were used to synchronize estrus and ovulation. The animals were divided into three equal groups (5 animals for each group). The 1st and 2nd group injected intravaneously after eCG injection with 1x10^8 and 2x10^8 respectively with BMSCs while the 3rd group was injected with normal saline which serve as a control group. Blood samples were collected during pregnancy, at day 10, 21, 85 from the jugular vein. At day 10, 21 the results showed that there was a significant difference (p≤0.05) in the level of progesterone between treated groups as compared with the control group. While there was no significant difference between different groups at day 85. It was concluded from this study that BMSCs have a beneficial effect in ewe’s reproductive system, by increasing the level of progesterone at early pregnancy.

Keywords: Bone marrow stromal cells, progesterone, pregnancy, ewes.
with chemotherapy by BMSCs showed that the ovarian structure and functions restored, i.e. It promote growth and function of corpus luteum in the ovaries that leads to more progesterone secretion (7–10). It has been reported that BMSCs differentiated into granulosa (11), endometrial (12,13) and endothelial cells (14) in animals. BMSCs regenerates new endometrial layer under the control of estrogen and progesterone (15). The aim of this study was to measure the levels of progesterone in pregnant ewes after treatments with a different doses of BMSCs.

Materials and Methods

This study was carried out on 15 ewes, 3-5 years old and 35-54 Kg., with adding two ewes under one year for bone marrow collection. The animals were raised at the farm of college of veterinary medicine, university of Fallujah, Fallujah, Al-Anbar province, during the period from May to November-2019. Flurogestone Acetate Sponges, (FAG) and 400 i.u. Equine chorionic gonadotropin (eCG), Syncropart were used to synchronize estrus and ovulation. Rams were introduced to the ewes immediately after sponge removed. The animals were divided into three equal groups. The 1st and 2nd group (T1, T2) injected intravenously after eCG injected with 1x10^8 and 2x10^8 respectively with BMSCs extracted from sheep sternum bone (16), and cultured in vitro according to Abd-allah et al., (2013) with some modifications, while the 3rd group where injected with normal saline which served as a control group. All ewes were brought indoors during pregnancy at day 10, 21, 85 for blood sampling. Samples of 10 ml blood were collected by jugular venepuncture into a gel clot activator vacutainer tube, blood left to clot at 4°C then centrifuged for 10 minutes at 3000 rpm. Serum were drawn off and stored at -20°C. Progesterone calculated via Enzyme Linked Immuno Sorbent Assay (ELISA) technique. “Statistical analysis: The data obtained were subjected to statistical analysis, using two way analysis of variance (ANOVA) and least significant differences (LSD) post hoc test was performance by using SPSS-24”.

Result and Discussion

Table-1- and figure -1- showed the level of progesterone in a pregnant Iraqi local breed ewes treated with BMSCs. The results showed that the concentration of progesterone at day 10 of pregnancy was 3.34±0.24 and 3.44±0.23 ng/ml in T1 and T2 respectively, while it was 2.33±0.09 ng/ml in T3. There was a significant difference (p≤0.05) in the level of progesterone in the T1 and T2 as Compared with the T3 (control group). At day 21 of pregnancy the result showed that the level of progesterone was 3.64±0.21 ng/ml in T1 and 3.84±0.10 ng/ml in T2, while it was 2.50±0.14 ng/ml in T3. There was a significant difference (p≤0.05) in the level of progesterone between treated groups T1, T2 as compared with the T3 control group. It has been observed that P4 (progesterone) play a role in maintaining of pregnancy and survival of the embryo and this occurs during maternal recognition of pregnancy (17) it has been reported that BMSCs could differentiated to granulosa cells which is the main Source of p4 from the ovaries during early embryonic life, before the placenta begins to Secrete p4 (11). So the increase levels of p4 in T1, T2 might be due to the action of BMSCs that promote granulosa cells formation & differentiation and secretion of P4 at day 10 and day 21. It’s also thought that P4 increase its level at early pregnancy to prevent early embryonic death especially under heat stress (17). It’s also suggested that BMSCs stimulate angiogenesis and folliculo-luteal transition (15). The results of the P4 level at day 85 were 9.76±0.34, 9.25±0.12 and 9.96±0.36 ng/ml for T1, T2 and T3 respectively. There was no significant difference between different groups in the level of P4. This might be due to the well development of placenta that secreted a large amount of P4 (18). Ricketts & Flint (1980) (19) reported that, after day 50 of gestation to the date of parturition in sheep, maintenance of pregnancy depends upon the
secretion of progesterone from placenta. Similar suggestion have been reported by several workers (7,15,20).

Table 1. Progesterone level in a pregnant Iraqi local breed ewes treated with BMSCs.

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Periods of gestation</th>
<th>Days of gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 10</td>
<td>Day 21</td>
</tr>
<tr>
<td>T1 (1x10^8)</td>
<td>3.34±0.24</td>
<td>3.64±0.21</td>
</tr>
<tr>
<td></td>
<td>A, b</td>
<td>A, b</td>
</tr>
<tr>
<td>T2 (2x10^8)</td>
<td>3.44±0.23</td>
<td>3.84±0.10</td>
</tr>
<tr>
<td></td>
<td>A, b</td>
<td>A, b</td>
</tr>
<tr>
<td>(control)</td>
<td>2.33±0.09</td>
<td>2.50±0.14</td>
</tr>
<tr>
<td></td>
<td>B, b</td>
<td>B, b</td>
</tr>
</tbody>
</table>

Different small letters indicate significant differences between the times within one row at (P≤0.05). Different capital letters refer significant differences between groups within one column at (P≤0.05).

Figure 1. Progesterone level in a pregnant Iraqi local breed ewes treated with BMSCs.

Conclusion

It was concluded from this study that BMSCs have beneficial effect in ewe’s reproductive system, by increasing the level of P4 at early pregnancy.

References


