Research Article

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Progesterone Levels During Pregnancy in Ewes Treated with Bone Marrow Stromal Cells

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Abstract

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 intravenously 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 ($p\leq0.05$) 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.

Keywards: Bone marrow stromal cells, progesterone, pregnancy, ewes.

تركيز هرمون البروجستيرون خلال الحمل للنعاج المعاملة بالخلايا الجذعية اللحمية لنخاع العظم

الخلاصة

أجريت الدراسة لمعرفة تأثير الخلايا الجذعية اللحمية لنخاع العظم (BMSCs) على تركيز هرمون البروجيستيرون اثناء الحمل في النعاج. تم توحيد الشبق بأستخدام الاسفنجات المهبلية المشبعة بمادة Flurogestone Acetate 40ملغم، مع الحقن العضلي لهرمون مصل الفرس الحامل eCG 400 وحدة دولية في وقت سحب الاسفنجات المهبلية. تم تقسيم حيوانات التجربة الى ثلاث مجاميع متساوية، بواقع خمس اناث لكل مجموعة. حقنت المجموعة الاولى والثانية وريدياً بالخلايا الجذعية اللحمية -بعد حقن هرمون eCG- وبواقع $1x10^8$ بالنسبة للمجموعة الاولى و $2x10^8$ للمجموعة الثانية، في حين عوملت المجموعة الثالثة بالمحلول الملحي الفسلجي وعدت كمجموعة سيطرة. تم سحب الدم من الحيوانات عن طريق الوريد الوداجي في الايام 10، 21، 85 من الحمل. أظهرت النتائج وجود فرق معنوي ($p\leq0.05$) لمستوى هرمون البروجستيرون بين مجموعتي المعاملة ومجموعة السيطرة خلال الايام 10، 21 من الحمل. في حين لم يظهر اي فرق معنوي بين اي من المعاملات خلال اليوم 85 من الحمل. استنتج من الدراسة، ان للخلايا الجذعية اللحمية لنخاع العظم تأثير معزز لمستوى هرمون البروجستيرون خلال الثلث الاول من الحمل والذي ينعكس بالنتيجة على رفع مستوى الخصوبة.

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

with chemotherapy by BMSCs showed that the ovarian structure and functions restored, i.e. It promote growth and function of corpus leutum 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 (T_1, T_2) 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 T_1 and T_2 respectively, while it was 2.33±0.09 ng/ml in T_3. There was a significant difference ($p\leq0.05$) in the level of progesterone in the T_1 and T_2 as Compared with the T_3 (control group). At day 21 of pregnancy the result showed that the level of progesterone was 3.64±0.21 ng/ml in T_1 and 3.84±0.10 ng/ml in T_2, while it was 2.50±0.14 ng/ml in T_3. There was a significant difference ($p\leq0.05$) in the level of progesterone between treated groups T_1, T_2 as compared with the T_3 control group. It has been observed that P_4 (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 p_4 from the ovaries during early embryonic life, before the placenta begins to Secrete p_4 (11). So the increase levels of p_4 in T_1, T_2 might be due to the action of BMSCs that promote granulosa cells formation & differentiation and secretion of P_4 at day 10 and day 21. It's also thought that P_4 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 P_4 level at day 85 were 9.76±0.34, 9.25±0.12 and 9.96±0.36 ng/ml for T_1, T_2 and T_3 respectively. There was no significant difference between different groups in the level of P_4. This might be due to the well development of placenta that secreted a large amount of P_4 (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.

Treated groups	Periods of gestation		
	Day 10	Day 21	Day 85
T1 ($1x10^8$)	3.34±0.24 A, b	3.64±0.21 A, b	9.76±0.34 a
T2 ($2x10^8$)	3.44±0.23 A, b	3.84±0.10 A, b	9.25±0.12 a
(control)	2.33±0.09 B, b	2.50±0.14 B, b	9.96±0.36 a

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

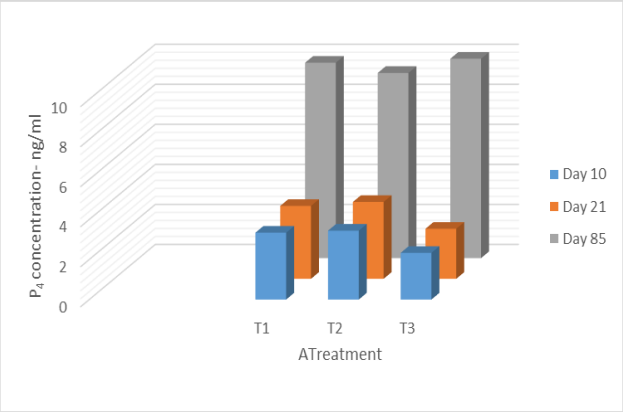

Figure 1. Progesterone level in a pregnant Iraqi local breed ewes treated with BMSCs.

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.

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