

**Progesterone Levels During Pregnancy in Ewes Treated with Bone Marrow Stromal Cells****A.A.Omar<sup>2\*</sup>, T.R. Mohammed<sup>1</sup> and Salah M.A.AL-Kubaisi<sup>2</sup>**<sup>1</sup>Animal Production, College of Agriculture, University of Anbar, Iraq; <sup>2</sup>College of veterinary medicine, University of Fallujah, IraqEmail: \*[alhadithyali@gmail.com](mailto:alhadithyali@gmail.com)Doi: <https://doi.org/10.37940/AJVS.2019.12.2.13>**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 1<sup>st</sup> and 2<sup>nd</sup> group injected intravenously after eCG injection with  $1 \times 10^8$  and  $2 \times 10^8$  respectively with BMSCs while the 3<sup>rd</sup> group where injected with normal saline which serve as control group. Blood samples were collected during pregnancy, at day 10, 21, 85 from jugular vein. At day 10, 21 the results showed that there was a significant difference ( $p \leq 0.05$ ) 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.****تركيز هرمون البروجستيرون خلال الحمل للنجاح المعاملة بالخلايا الجذعية اللحمية لنخاع العظم****الخلاصة**

أجريت الدراسة لمعرفة تأثير الخلايا الجذعية اللحمية لنخاع العظم (BMSCs) على تركيز هرمون البروجستيرون أثناء الحمل في النعاج. تم توحيد الشبق باستخدام الاسفنجيات المهبلية المشبعة بمادة 40mg Flurogestone Acetate، مع الحقن العضلي لهرمون مصل الفرس الحامل 400 eCG وحدة دولية في وقت سحب الاسفنجيات 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  $1 \times 10^8$  بالنسبة للمجموعة الاولى و  $2 \times 10^8$  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 ( $p \leq 0.05$ ) 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 system includes maintenance of pregnancy,

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 method

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 1<sup>st</sup> and 2<sup>nd</sup> group (T<sub>1</sub>, T<sub>2</sub>) injected intravenously after eCG injected with  $1 \times 10^8$  and  $2 \times 10^8$  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 3<sup>rd</sup> 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  $3.34 \pm 0.24$  and  $3.44 \pm 0.23$  ng/ml in T<sub>1</sub> and T<sub>2</sub> respectively, while it was  $2.33 \pm 0.09$  ng/ml in T<sub>3</sub>. There was a significant difference ( $p \leq 0.05$ ) in the level of progesterone in the T<sub>1</sub> and T<sub>2</sub> as Compared with the T<sub>3</sub> (control group). At day 21 of pregnancy the result showed that the level of progesterone was  $3.64 \pm 0.21$  ng/ml in T<sub>1</sub> and  $3.84 \pm 0.10$  ng/ml in T<sub>2</sub>, while it was  $2.50 \pm 0.14$  ng/ml in T<sub>3</sub>. There was a significant difference ( $p \leq 0.05$ ) in the level of progesterone between treated groups T<sub>1</sub>, T<sub>2</sub> as compared with the T<sub>3</sub> control group. It has been observed that P<sub>4</sub> (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<sub>4</sub> from the ovaries during early embryonic life, before the placenta begins to Secrete p<sub>4</sub> (11). So the increase levels of p<sub>4</sub> in T<sub>1</sub>, T<sub>2</sub> might be due to the action of BMSCs that promote granulosa cells formation & differentiation and secretion of P<sub>4</sub> at day 10 and day 21. It's also thought that P<sub>4</sub> 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<sub>4</sub> level at day 85 were  $9.76 \pm 0.34$ ,  $9.25 \pm 0.12$  and  $9.96 \pm 0.36$  ng/ml for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. There was no significant difference between different groups in the level of P<sub>4</sub>. This might be due to the well development of placenta that secreted a large amount of P<sub>4</sub> (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

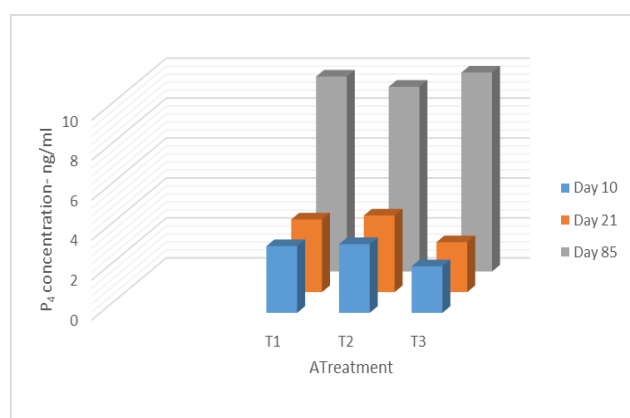
Suggestion have been reported by several workers (7,15,20).

**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<br>(1x10 <sup>8</sup> ) | 3.34±0.24<br>A, b    | 3.64±0.21<br>A, b | 9.76±0.34<br>a |
| T2<br>(2x10 <sup>8</sup> ) | 3.44±0.23<br>A, b    | 3.84±0.10<br>A, b | 9.25±0.12<br>a |
| (control)                  | 2.33±0.09<br>B, b    | 2.50±0.14<br>B, b | 9.96±0.36<br>a |

Different small letters indicate significant differences between the times within one raw at ( $P \leq 0.05$ ).

Different capital letters refer significant differences between groups within one column at ( $P \leq 0.05$ ).



**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

1. Alawi IK, Mohammed TR, Majeed AF. Effect of Arginine and Selenium with Vitamin E on WBC and the level of hormones in Iraqi ewes discharged. *Al-Anbar J Vet Sci.* 2019;12(1):6–12.
2. Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front Biosci.* 2002;7(7):d1879--d1898.
3. Ibrahim NS, Nawaf SA. Progesterone and Ultrasound Comparative Study of Estrus and Pregnancy between induced estrus and normal cyclic Dogs. *Al-Anbar J Vet Sci.* 2016;9(2):24–30.
4. Tuckey RC. Progesterone synthesis by the human placenta. *Placenta.* 2005;26(4):273–81.
5. Arck P, Hansen PJ, Mulac Jericevic B, Piccinni M-P, Szekeres-Bartho J. Progesterone during pregnancy: endocrine--immune cross talk in mammalian species and the role of stress. *Am J Reprod Immunol.* 2007;58(3):268–79.
6. Lauretta R, Sansone M, Sansone A, Romanelli F, Appetecchia M. Gender in endocrine diseases: Role of sex gonadal hormones. *Int J Endocrinol.* 2018;2018.
7. Abd-Allah SH, Shalaby SM, Pasha HF, Amal S, Raafat N, Shabrawy SM, et al. Mechanistic action of mesenchymal stem cell injection in the treatment of chemically induced ovarian failure in rabbits. *Cytherapy.* 2013;15(1):64–75.
8. Badawy A, Sobh MA, Ahdy M, Abdelhafez MS. Bone marrow mesenchymal stem cell repair of cyclophosphamide-induced ovarian insufficiency in a mouse model. *Int J Womens Health.* 2017;9:441.
9. Fu X, He Y, Wang X, Peng D, Chen X, Li X, et al. Overexpression of miR-21 in stem cells improves ovarian structure and function in rats with chemotherapy-induced ovarian damage by targeting PDCD4 and PTEN to inhibit granulosa cell apoptosis. *Stem Cell Res Ther.* 2017;8(1):187.
10. Mohamed SA, Shalaby SM, Abdelaziz M, Brakta S, Hill WD, Ismail N, et al. Human mesenchymal stem cells partially reverse infertility in chemotherapy-induced ovarian failure. *Reprod Sci.* 2018;25(1):51–63.
11. Besikcioglu HE, Saribas GS, Ozogul C, Tiryaki M, Kilic S, Pinarli FA, et al. Determination of the effects of bone

- marrow derived mesenchymal stem cells and ovarian stromal stem cells on follicular maturation in cyclophosphamide induced ovarian failure in rats. *Taiwan J Obstet Gynecol.* 2019;58(1):53–9.
12. Liu Y, Tal R, Pluchino N, Mamillapalli R, Taylor HS. Systemic administration of bone marrow-derived cells leads to better uterine engraftment than use of uterine-derived cells or local injection. *J Cell Mol Med.* 2018;22(1):67–76.
  13. Gao L, Huang Z, Lin H, Tian Y, Li P, Lin S. Bone marrow mesenchymal stem cells (BMSCs) restore functional endometrium in the rat model for severe Asherman syndrome. *Reprod Sci.* 2019;26(3):436–44.
  14. Tepper OM, Sealove BA, Murayama T, Asahara T. Newly emerging concepts in blood vessel growth: recent discovery of endothelial progenitor cells and their function in tissue regeneration. *J Investig Med.* 2003;51(6):353–9.
  15. Zhao Y, Chen S, Su P, Huang F, Shi Y, Shi Q, et al. Using Mesenchymal Stem Cells to Treat Female Infertility: An Update on Female Reproductive Diseases. *Stem Cells Int.* 2019;2019.
  16. Grunsell CS. Marrow Biopsy in Sheep: I. Normal. *Br Vet J.* 1951;107(1):16–23.
  17. Noakes DE, Parkinson TJ, England GCW. The caesarean operation and the surgical preparation of teaser males. *Vet Reprod Obstet 9th Ed Saunders, Elsevier*, pp-347-366. 2009;
  18. Pineda MH, Dooley MP, others. McDonald's veterinary endocrinology and reproduction. Iowa state press; 2003.
  19. Ricketts AP, Flint APF. Onset of synthesis of progesterone by ovine placenta. *J Endocrinol.* 1980;86(2):337–47.
  20. Wei X, Yang X, Han Z, Qu F, Shao L, Shi Y. Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin.* 2013;34(6):747–54.