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Effect of using Bone Marrow Stromal Cells on Uterine Involution in Iraqi Ewes

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ABSTRACT
During involution the uterus in ewes undergoes a clear remodeling; unfortunately, information on cellular and molecular activities that controls this interval is inadequate. The working hypothesis of this study applied in, is that uterus returns to a non-pregnant status during involution, involves the proliferative and regeneration activity of mesenchymal stem cells (MSCs) which migrated to the uterus servomechanism that regulates and remodeling endometrial gland epithelial surface postpartum. The present study consist of 15 Iraqi local breed ewes were divided equally into three groups. The 1st and 2nd group were injected intravenously with bone marrow stromal cells (BMSCs) in a doses 1×10^8, 2×10^8 respectively, 48 hours postpartum, while the 3rd group served as a control group. Uterine biopsy were taken at 7, 15, 17 and 21 days postpartum, and processed to histopathological examination. The progressive of involution was clearly observed in treated groups till ended at days 21 for 2nd group, as well as in the 1st group but in a lesser extent. It was concluded that BMSCs has a beneficial effect on uterine involution which is completed at day 21in Iraqi ewes.

Keywords: Bone marrow, stem cells, histopathological examination, uterine involution, ewes, BMSCs.

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INTRODUCTION
Stem cells are undifferentiated cells having the ability to differentiate into specific cell types[1]. The source of these cell can be found in umbilical cord blood, umbilical cord tissue, bone marrow, fat tissue and other somatic stem cells[2]. Stem cell have been isolated or described from ovine endometrium[3]. It plays an important role in the repair process of tissue damage[4]. The uterus is a highly regenerative tissue undergoes growth and apoptosis postpartum especially at uterine involution[5]. Ovine postpartum periods include the duration from parturition (Lambing) to the onset of cyclic ovarian activity that allow the next pregnancy to occur in a short period[6]. The uterine involution includes three events; reduction in uterine size, losses of tissues and finally repair of tissue[7]. It has been reported that macroscopic uterine involution in the ewes ranged between 17 to 35 days[8,9]. Histological change during postpartum in uterine involution have been described[7]. There is a little information about the effect of stem cell on ovine uterine involution. Therefor this study was aimed to demonstrate the effect of BMSCs on microscopic uterine involution in Iraqi local breed ewes.

MATERIALS AND METHODS
The study was conducted on 15 Iraqi local breed ewes aged between 3 to 5 years and weighted 35-54 Kg., additionally two ewes aged less than one year were used for bone marrow samples extraction presented at the farm of college of veterinary medicine, university of Fallujah. The animals were divided into three groups, each group included 5 ewes. The 1st group were injected intravenously with 1×10^8 BMSCs extracted from ovine sternum bone marrow according to[10], and were cultured in vitro according to[11]. The 2nd group were injected intravenously with 2×10^8 BMSCs which extracted and cultured from the same source of the 1st group. The 1st and 2nd groups injected with BMSCs at 48 hours postpartum, while the 3rd group served as a control group were injected intravenously with normal saline at the same time. Uterine biopsy were taken after laparotomy from uterine horns at 7, 15, 17 and 21 days postpartum. The samples were put in natural puffer formalin buffer solution 10% and processed according to[12]. Hematoxylin and Eosin were used for staining of the histopathological slides then they examined under light microscope.

RESULT AND DISSECTION
It has been reported that uterine involution in the ewes occurred between 17-30 days postpartum [6]. Uterine involution occurred for preparation of the genital system to the estrus cycle and next pregnancy. These preparations include reduction in the size of the uterus, tissue losses and tissue repair [7]. Stem cells were used to treat several tissues [13–15], and its play an important roles in the regeneration and tissue repair during uterine involution [16]. The endometrial repair are similar to usual trauma repair processes in other tissues, which involves usually three stages of inflammation, tissue repair, and remodeling of tissue[17], where after the injury of endometrium due to the normal or abnormal parturition, the epithelial cells of endometrium are suffering from some damages which result in loss of endometrial coverage of the uterine cavity, and endometrial fibrosis will occurred due to interstitial cell hyperplasia, adhesion and inhibition of proliferation[18], where the severe uterine injury leads to proliferation of interstitial fibrous connective tissue to repair the damaged of uterine tissue, and thus resulting in fibrotic lesions. Therefore, the level of endometrial damage can be detected by extent of endometrial fibrosis[19]. In the present study, the therapeutic ability of BMSCs were used to ameliorate the uterine involution, in term of, endometrial injury repaired by a single dose of 1×10^8 MSCs in the first group and 2×10^8 MSCs in the second group. To ascertain the postpartum therapeutic effects of BMSCs on ovine uterus, the animals of treatment groups compared with the control group in which the ewes revealed several damages in the uterus, while in the 1st and 2nd treatment groups, these damages were repaired 21 and 14 days after transplantation of MSCs, respectively, so these results are in agreement with the results of[20] who found that MSCs can ameliorate endometrial and muscle proliferation. Histological picture of

uterus for control group 17 days postpartum (Fig-1) showed low columnar epithelium, the epithelial lumen also seen rufled, the endometrial gland is narrow, the endometrial and myometrial width increased slightly. Similar observation have been made by[21,22].

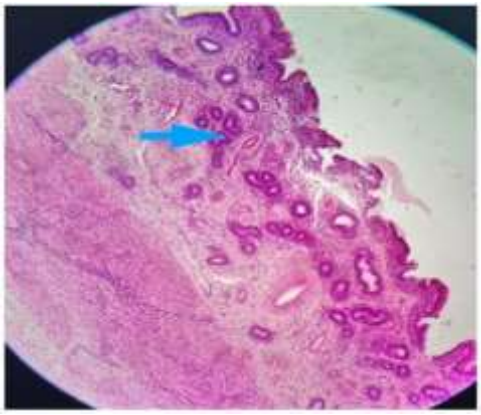

Fig. 1: Histophotographic image from control group at day 17 postpartum showed low columnar epithelium, the epithelial lumen also seen rufled, the endometrial gland is narrow, the endometrial and myometrial width increased slightly (arrow). H&E, ×10.

Histological picture of treated ewes with 1×10^8 BMSCs at day 7 showed increase thickness of muscle layer (myometrium) with fibroplasia (Fig-2). This changes might be due to fact that stem cells are responsible for continuous regeneration and cell production[22]. These result are in agreement with[23,24].

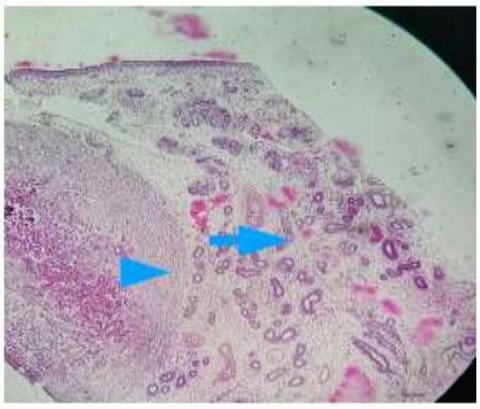

Fig. 2: Histophotographic image from treated group with 1x108 MSCs at day 7 showed increase the thickened of myometrium (arrow) with increase fibroplasia (arrow head). H&E, ×10.

Microscopic examination of treated ewes with 2×10^8 MSCs at day 7 showed an active proliferative changes of endometrial gland epithelial surface (Fig-3).

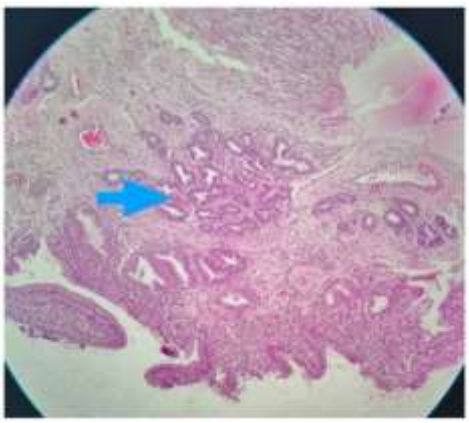

Fig. 3: Histophotographic image from treated group with 2×108 MSCs at day 7 showed a proliferative active endometrial gland epithelial surface (arrow). H&E, ×10.

This picture showed the more progressive uterine involution with this dose of MSCs as compared with control or other dose (1×10^8) of BMSCs. It has been reported that stem cells and $PGF_{2}\alpha$ may be responsible for activity of the mediator of Re-epithelization and regeneration process of endometrium [16]. It is also indicated that uterine tissue regeneration occurred in the uterine caruncular and intercaruncular areas. This tissue regeneration have not shown an apoptosis. This might be due to fragmentation of the DNA which was observed only in large vacuolated cells around endometrial glands till day 7 in a study performed by[7]. Histological exam revealed a significant regression of endometrial glands and flattened endometrial epithelial surface in treated ewes with 1×10^8 BMSCs at day 15 (Fig-4).

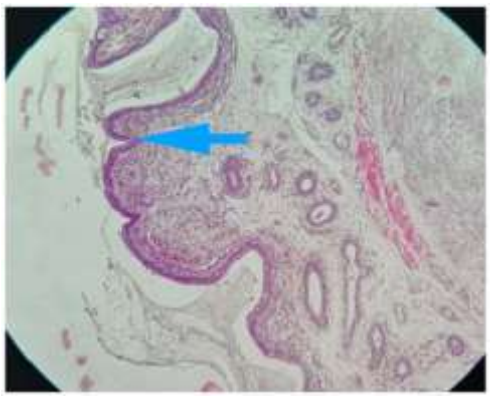

Fig. 4: Histophotographic image from treated group with 1×108 BMSCs at day 15 showed a significant regress in the endometrial glands and flatted endometrium surface (arrow). H&E,×10.

During involution process, there were a frank reduction in blood vessels observed in curuncular and intercuruncular areas at the uterine wall. These results might be due to effect of stem cells that increase the repair of uterine wall as compared with control untreated ewes [6]. It has been found that uterine involution in ewes was completed at day 17 postpartum however the involution was delayed in ewes that suffer from obstetrical problems such as dystocia, retention

of fetal membranes, uterine prolapse, metritis and caesarean section [8,25].

(Fig-5) showed the microscopic picture of the uterine wall in ewes treated with 2x10^8 MSCs at day 15 there is a decrease and reduction of endometrial layer with well distinct stratum compactum and stratum spongiosum. There were a high marked reduction in the vascularity of caruncular and intercuruncular area as compared with control and ewes treated with 1×10^8 BMSCs. This might be due to stem cells in this dose stimulate cell production, proliferation and regeneration process[22]. Microscopic exam (Fig-6) of ewes treated with 2×10^8 BMSCs at day 21 showed complete epithelial proliferation of endometrium with great narrowing of blood vessels. The uterine wall layers were in a state similar to that before pregnancy. Similar observation showed in ewes treated with 1×10^8 BMSCs but to a lesser extent. These findings in accordance to[8,16].

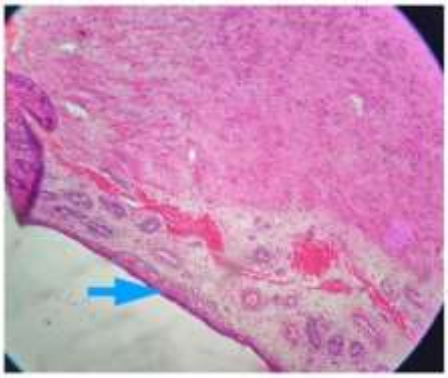

Fig. 5: Histophotographic image from treated group with 2×108 BMSCs at day 15 showed a decrease endometrial layer size with well-defined stratum compactum and stratum spongiosum (arrow). H&E, X10.

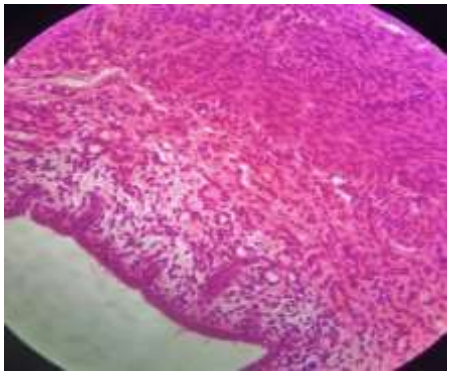

Fig. 6: Histophotographic image from treated group with 2×108 BMSCs at day 21 showed complete epithelial proliferation of endometrium with great narrowing of blood vessels and no clear pathological changes observed on uterus layers. H&E, × 40.

CONCLUSION

It was concluded from this study that MSCs injected i.v. in a dose of 2x10^8 has a beneficial effect on uterine involution and it was completed at day 21 in local Iraqi ewes breed.

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