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Effect of exogenous oxytocin on the expression of oxytocin receptor gene and uterine involution in local Iraqi cows

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Abstract

The present work aimed to study the effect of exogenous oxytocin injection on the expression of the oxytocin receptor gene and the duration of uterine involution in the local Iraqi breed of Karradi cows. Twenty cows were divided into two groups. The first group was considered a control; the second group was injected with 100 IU/IM of oxytocin twice weekly for four weeks postpartum. The uterine involution velocity was monitored using ultrasonography by measuring the endometrium thickness, ovarian diameter, cervix diameter, uterine horns diameter, serum progesterone, and estrogen levels by indirect ELISA, and the expression of oxytocin receptor gene was monitored by conventional PCR. The result of the treated group showed that the progesterone concentration was significantly decreased. The estrogen concentration was significantly increased. Moreover, the endometrium thickness was significantly decreased in the second, third, and fourth weeks, also, the ovarian diameter was significantly decreased in the first and second weeks, but it has significantly increased in the fourth week. In addition, the cervix diameter was significantly decreased in the first and second weeks, and the uterine horns diameter was significantly decreased in all weeks compared to the control group at $P<0.05$. The findings of the PCR study explain a substantial link between rapid uterine involution and *OTXRs* gene overexpression, where *OTXRs* gene expression was increased in the oxytocin group in comparison with the control group. This result was present in a coordinated manner with the result of the estrus cycle and ovarian reactivation. We conclude that injection of exogenous oxytocin at 100 IU/IM twice weekly for four weeks postpartum will increase the expression of the oxytocin receptor gene, leading to a decrease in the duration of uterine involution. Accelerate the occurrence of estrus in the local Iraqi breed of Karradi cows.

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Introduction

Rapid uterine involution and conception after parturition are essential to increase the economic income on the cow's farms. Progressive economic losses can be attributed to prolonging the postpartum period and failure or delay in conception (1). Rapid resumption of the ovarian activity led to increasing uterine involution ovarian function during the postpartum period leading to a decrease in uterine involution duration, rapid cyclicity, then pregnancy (2). Uterine involution can refer to the process of a uterus returning to its previous size, which it is takes about 40 to 50 days after calving in normal conditions (3,4). Uterine involution has an intense physiological process that includes endometrial regeneration, decreased uterine blood flow, decreased endometrial vascularity (3), and decreased muscle mass,

which all shrink the uterus to its size before pregnancy (5,6). Uterine involution is started by the action of prostaglandins hormone produced and excreted by the uterus; prostaglandins trigger the expulsion of placental residues and cause a reduction in the size of uterine muscles (2). Oxytocin plays a vital role in uterine involution, which acts on the muscle fiber in the uterus wall, causing them to contract in the physiological process end by the decrease in the size of the uterus (4). The oxytocin receptor gene (OTX) has an essential role in cows' uterine involution and reproductive tract (7). The *OTXRs* was composed of 389 polypeptides and had seven transmembrane domains that belong to class- I of the G-protein receptor (8). The *OTXRs* has been shown to play an important role in addition to factor genes in uterine involution, which is highly expressed in the last stage of pregnancy and returns to its previous status after calving (9).

The present work aimed to study the effect of exogenous oxytocin injection on the expression of *OTXRs* and the duration of uterine involution in a local Iraqi breed of Karradi cows.

Materials and Methods

Animals

The study was conducted in a field consisting of 600 cows distributed in six stations. The field is located in the district of Hamdaniya / Sheikh Omar. A total of thirty postpartum local breed cows were aged (3-6years) with an average bodyweight of (300-500 kg) with a history of one calvining at least. They were housed in semi-opened shade regarding the nutritional regime. Careful clinical examination was done to determine that animals are healthy and free from diseases. Before starting the experiment, all cows were submitted to trans-rectal and trans-abdominal ultrasonography to make sure that the 20 local cows were in advance pregnancy. The first group was considered a control group, while the second group was injected with oxytocin 100 IU/IM (10 ml, Interchemte, Holland) twice weekly from 3 days after parturition for four weeks postpartum period.

Parameters

The uterine involution velocity was monitored by using ultrasonography by measuring the endometrium thickness (mm), ovarian diameter (mm), cervix diameter (mm), and uterine horns diameter (mm) (10), in addition to estimating the level of serum progesterone (ng/ml) and serum estrogen (pg/ml) by indirect ELISA (MonoBind, USA) (11). All parameters were recorded twice weekly, from 3 days after parturition to four weeks postpartum. Before the ultra-sonographic examination, a process to localize the cervix and uterine horns was applied by rectal palpation (12); after that, the transrectal transducer was introduced into the rectum to measure the endometrium thickness, ovarian diameter, cervix diameter, and uterine horns diameter (13).

OTXRs primer

The *OTXRs* primers used in the current study forwarding 5'-GCATGTTCGCGTCCACCTACCT-3', while at reverse 5'-CCCGTGAAGAGCATGTAGATCC-3', and the product size was 634 bp (14).

Blood sampling and DNA extraction

Blood samples were collected twice weekly for four weeks postpartum in a particular test tube with an anticoagulant. The whole blood plasma was used to purify the DNA using the specific extraction kit (Quick-DNA™ Blood MiniPrep, USA).

PCR protocol and agarose gel electrophoresis

To identify the OTX, a 2% agarose gel (Tris-Borate EDTA, Conda, USA). Later, the DNA samples with SiZer were uploaded to wells then the procedures for electrophoresis were initiated at 5v/cm for 90 minutes to separate the DNA, stained with red safe nucleic acid stain (Intron, Korea). The PCR procedure program was applied as follows, in the first stage, the denaturation for 30 seconds at 94°C for one cycle was applied. In the second stage, denaturation for 3 seconds at 94°C, annealing for 30 seconds at 65°C, and extension for 30 seconds at 72°C. All these steps were applied at 35 cycles. In the third stage, the extension was applied for 60 seconds at 72°C for one cycle (15). at the end of this protocol, the UV illuminator was used to visualize the DNA band.

Statistical analysis

The means included in the current study were analyzed using one-way ANOVA to compare different weeks in the same group, and paired t-tests were used to compare the differences between the treated group and control group at the same week at $P<0.05$.

Results

Progesterone hormone

The current study showed that the progesterone level in both control and oxytocin groups decreased significantly with the progress of experimental weeks, which means the presence of significant decreasing differences between different weeks in the same group. Progesterone concentration had significantly decreased from the 1st to the fourth week during the postpartum period for both groups of animals. Moreover, it should be noted that the exogenous oxytocin had a significant effect on the level P4 (Table 1). Oxytocin decreased the level of P4 significantly from the first week to the fourth week from a week to 4 weeks during the postpartum period.

Table 1: Serum progesterone in cows' postpartum

	Progesterone concentration (ng/ml) ± SE	
	Control group	Oxytocin group
1st week	[a] 3.218±0.388	[a] 2.393±0.230 *
2nd week	[b] 2.248±0.161	[b] 1.943±0.063 *
3rd week	[c] 1.128±0.089	[c] 0.824±0.020 *
4th week	[d] 0.618±0.017	[d] 0.359±0.128 *

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group at P<0.05.

Estrogen hormone

The current study showed that the estrogen concentration had significantly increased from the 1st to the fourth week during the postpartum period for both groups of animals. Moreover, it should be noted that the exogenous oxytocin had a significant effect on the level E2 (Table 2). Oxytocin increased the level of E2 significantly from the first week to the fourth week from a week to four weeks during the postpartum period experiment in both control and oxytocin groups. With attention to that, the estrogen level was increased in the oxytocin group more rapidly than that recorded in the control group.

Table 2: Serum estrogen in cows' postpartum

	Estrogen concentration (pg/ml) ± SE	
	Control group	Oxytocin group
1st week	[c] 43.09±0.64	[d] 48.21±2.81
2nd week	[c] 47.56±3.72	[c] 61.91±5.12 *
3rd week	[b] 77.32±7.67	[b] 118.91±13.21 *
4th week	[a] 101.75±2.54	[a] 164.51±6.72 *

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group.

Endometrium thickness

The result of the current study showed that the endometrium thickness in both control and oxytocin groups was decreased significantly with the progress of experimental weeks, which means the presence of significant decreasing differences between different weeks in the same group, the endometrium thickness had significantly decreased from the 1st to the fourth week during the postpartum period for both groups of animals. Moreover, it should be noted that the exogenous oxytocin had a significant effect on the endometrium thickness (Table 3). Oxytocin decreased the endometrium thickness significantly from the first week to the fourth week from a week to 4 weeks during the postpartum period. With attention to that, the endometrium thickness was decreased in the oxytocin group more rapidly than that recorded in the control group.

Table 3: Endometrium thickness in cows' postpartum

	Endometrium thickness (mm) ± SE	
	Control group	Oxytocin group
1st week	[a] 42.19±2.37	[a] 40.68±2.86
2nd week	[b] 34.15±1.41	[b] 19.94±0.51 *
3rd week	[c] 27.06±0.70	[b] 17.26±0.95 *
4th week	[d] 22.48±1.75	[c] 13.88±0.93 *

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group.

Ovarian diameter

The current study showed that the ovarian diameter in the control group decreased significantly with the progress of experimental weeks, in which the ovarian diameter was at the diameter in the first week and decreased gradually to be at the lowest diameter in the fourth week in the control group. While in the oxytocin group, the ovarian diameter was at its highest significant diameter in the first week, then decreased in the second week and recorded at the lowest effective diameter in the third week. Later, in the fourth week, the result recorded a significant increase in ovarian diameter in the oxytocin group, indicating the reactivation of the ovary and starting a new estrus cycle in cows of this group. Comparing the ovarian diameter in the third week between the control and oxytocin groups showed that the ovarian diameter was not significantly different in the oxytocin and control groups in the third week. At the same time, it was observed that in the first and the second weeks, the ovarian diameter was significantly decreased in the oxytocin group than that recorded in the control group, which means the ovarian diameter was decreased significantly in the oxytocin group in comparison with control.

In contrast, in the fourth week, the ovarian diameter in the oxytocin group was significantly higher than that recorded in the control group (Table 4). In short lines, the ovarian diameter was decreased with the progress of time of the experiment in both control and oxytocin groups. With attention to that, the ovarian diameter was decreased in the oxytocin group more rapidly than that recorded in the control group, except in the fourth week, where it was significantly higher in the oxytocin group due to reactivation of the ovary and starting a new estrus cycle.

Cervix diameter

The result of cervix diameter in both control and oxytocin groups was decreased significantly with the progress of experimental weeks, which means the presence of significant decreasing differences between different weeks in the same group, cervix diameter had significantly decreased from the 1st to the fourth week during the postpartum period for both groups of animals. Moreover, it should be noted that the exogenous oxytocin had a significant effect on the cervix

diameter. Oxytocin decreased the cervix diameter significantly from the first week to the fourth week from a week to 4 weeks during the postpartum period. The result of cervix diameter was continued to be recorded in the treated group with oxytocin at the same patterns in the control group (Table 5).

Table 4: Ovary diameter in cows' postpartum

	Ovary diameter (mm) ± SE	
	Control group	Oxytocin group
1st week	[a] 42.05±3.88	[a] 34.51±1.43 *
2nd week	[b] 30.63±1.07	[c] 20.38±1.01 *
3rd week	[c] 24.54±1.57	[b] 24.861.18
4th week	[d] 13.92±1.40	[b] 25.64±1.28 *

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group.

Table 5: Cervix diameter in cows' postpartum

	Cervix diameter (mm) ± SE	
	Control group	Oxytocin group
1st week	[a] 45.18±1.91	[a] 37.41±1.42 *
2nd week	[a] 42.76±0.69	[b] 28.49±1.21 *
3rd week	[b] 23.14±0.86	[c] 22.78±0.87
4th week	[c] 20.82±0.78	[d] 19.94±0.51

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group.

Uterine horns Diameter

The result of the current study showed that the uterine horns diameter in both control and oxytocin groups was decreased significantly with the progress of experimental weeks, which means the presence of significant decreasing differences between different weeks in the same group, the uterine horns diameter had significantly decreased from the 1st to the fourth week during the postpartum period for both groups of animals. Moreover, it should be noted that the exogenous oxytocin had a significant effect on the uterine horn's diameter (Table 6). Oxytocin decreased the cervix diameter significantly from the first week to the fourth week from a week to 4 weeks during the postpartum period. The uterine horns diameter was decreased in the oxytocin group more rapidly than in the control group.

PCR for *OTXRs* gene

The result of conventional PCR techniques for detecting extracted DNA of *OTXRs* revealed bands of the nucleic acid of *OTXRs* gene at 634 bp from control and oxytocin groups at four weeks. The PCR result showed that the *OTXRs* was identified in 40, 50, 70 and 70% in the first, 2nd, third and fourth weeks in the control group (Figure 1), respectively, while the *OTXRs* was identified in 90, 90, 100, and 100% in 1st, 2nd 3rd and 4th weeks in oxytocin group (Figure 2) respectively (Table 7).

Table 6: Uterine horns diameter in cows' postpartum

	Uterine horns diameter (mm) ± SE	
	Control group	Oxytocin group
1st week	[a] 65.53±2.34	[a] 38.96±1.63 *
2nd week	[b] 43.68±1.50	[b] 26.67±1.07 *
3rd week	[c] 36.68±1.72	[c] 20.93±0.71 *
4th week	[d] 29.67±0.64	[d] 15.06±1.47 *

* Mean significant differences between oxytocin and control groups. Different vertical letters mean significant differences between weeks within the same group.

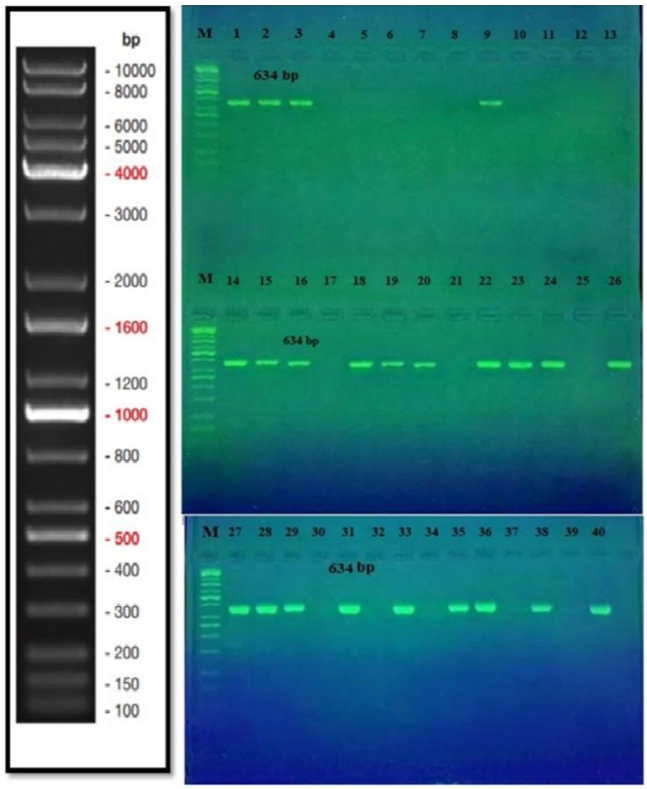

Figure 1: Agarose gel electrophoresis of PCR products for the control group. The positive result for the *OTXRs* gene at 634 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2, 1x TBE buffer for 1:30 hours, N: DNA ladder (100), *OTXRs* gene for 1-4 weeks.

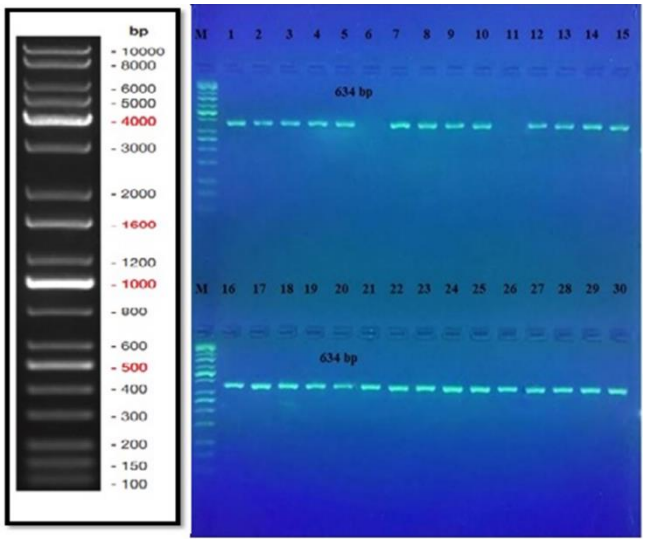

Figure 2: Agarose gel electrophoresis of PCR products for oxytocin group. The positive result for the *OTXRs* gene at 634 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2, 1x TBE buffer for 1:30 hours, N: DNA ladder (100), *OTXRs* gene for 1-4 weeks.

Table 7: *OTXRs* expression during different weeks

	OTXRs gene expression [n (%)]	
	Control group	Oxytocin group
1st week	4 (40%)	9 (90%)
2nd week	5 (50%)	9 (90%)
3rd week	7 (70%)	10 (100%)
4th week	7 (70%)	10 (100%)

Discussion

Domestic animals, especially dairy cows, are adapted to high production systems, in which postpartum recovery, a complex process through which the tubular genital shrink to reverse the hypertrophy caused by pregnancy, is known as uterine involution (16). The period from calving to clinically complete ovary rebound and returning the position and size of the uterus after parturition in the experimental cow is called the postpartum period. This period is affected by many factors such as age, season, reproductive status, milking, nutrition, and hormones (17). This period extends from 42 to 60 days after parturition (18). As a result, uterine involution was delayed by dystocia or any reproductive disorder after parturition (19,20).

In the current study, progesterone serum concentration in the cow of the control group was at the highest significant concentration in the first week and then started to decline in the second, third, and fourth weeks. These results agree with Rostami *et al.* (21) and Garcia - Ispierto *et al.* (22). This decline was caused by the natural physiological regression of pregnant corpus luteum by the proteolytic action of PGF2α that is secreted from the uterine endothelium after parturition. This action led to a decline in the progesterone concentration in the peripheral blood because the corpus luteum is the primary source of this hormone during the pregnancy (23). On the other hand, the progesterone serum level in a cow in the treated groups with oxytocin was the highest significant level in the first week of the experiment and decreased significantly in the following four weeks. These results were shown to agree with Bishop (24). During the last stage of pregnancy and parturition, the oxytocin and oxytocin receptor has a different action to upregulated the epithelial cell of the endometrium to syntheses and release the PGF2α, this increases in PGF2α secretion cause luteolysis of corpus luteum leads to a significant decrease in progesterone level in comparison with the control group (25,26).

In the current study, the estrogen serum concentration in the control group showed no significant differences between 1st week and the second week. This can be explained by progesterone's action, which causes negative feedback on the hypothalamus that prevents GnRH release, which leads to a decrease in releasing FSH and LH, which causes a stopping in ovarian follicle growth, leading to a decrease in estrogen release. There was a significant increase in estrogen levels in the 3rd and fourth weeks. This can be related to the action of PGF2α that caused luteolytic action on corpus luteum, that caused a decrease in progesterone level, which allowed to stimulating the hypothalamus to increase releasing GnRH, that cause increased secretion of FSH and LH, which together cause growing of ovarian follicles which cause to increase in estrogen level (27). On the other hand, the estrogen level in the first week did not express any significant differences between the control group and the treated group. In contrast, the estrogen level in the treated group was highest significantly compared to the control group in the next three weeks. The high level of estrogen here can be explained by the action of exogenous oxytocin that stimulates endothelial cells in the endometrium to release the PGF2α, leading to an increase in regression of corpus luteum and decrease in progesterone level in peripheral blood and causing the hypothalamus to release the GnRH that led to start a new cycle and cause an increase in estrogen serum level (28).

Ultrasonographic examination indicated that a complete uterine involution lasted six weeks after normal parturition (4,29). In this study, the endometrium thickness in the treated group during the fourth week increased significantly compared to the control group. This result is similar to the study conducted by Abdel-Khalek *et al.* (30). While the first week in both groups, there were non-significant differences between treated and control groups because the size of the uterus after parturition in cows was shrunk dramatically

during involution in the first ten days later it is followed by uterine tone this will lead to the shirking of the uterine in the next 10-14 days after calving (30).

The measurement of ovarian diameter in the control group showed a highly significant diameter in the first week of experimental and began to decrease significantly in the second and third weeks. This is related to the action of PGF2α that has the proteolytic effect on the corpus luteum, which causes a decrease in the size of ovarian diameter, while in the fourth week, the ovary rebound activity begins and starts a new cycle that causes an enlargement in size due to the growing follicles, those results showed to agree with (31). On the other hand, the result showed that the ovarian diameter in oxytocin groups was significantly high compared to the control group in the first, second, third, and fourth weeks. This direct action of oxytocin can explain this increased release of PGF2α. This increase led to the stop of progesterone action and began a new cycle and reactivation of the ovary to stimulate growing new follicles. This result is inconsistent with the study conducted by Anatoli *et al.* (23) and Yindee *et al.* (33).

As for the cervix diameter, the result showed a highly significant difference between the control group and the treated group during the first and second weeks, which agreed with the result obtained by Romoun *et al.* (34). on the other hand, the third and fourth weeks did not significantly differ between the treated and control groups. This can be explained by the contracting effect of oxytocin on cervix muscle fiber with an increase in estrogen level (35).

The uterine horn diameter significantly differed between the treated and control groups in all weeks. These results conform with the study conducted by El-Wishy *et al.* (36) and Alagar *et al.* (37). This is due to the physiological effect of myometrium after parturition and the contractile effect of oxytocin on the muscle fibers. An increase in estrogen secretion is vital in returning the uterus and cervix to standard size and average position.

The oxytocin receptor gene has a relationship with the last stage of parturition and postpartum periods, in which these stages affect and are influenced by the *OTXRs* gene (38). On the other hand, the uterus is considered the effector organ for both oxytocin and oxytocin receptor genes, where they act as potent uterotonic agents. They function to facilitate the parturition and uterine involution after calving (39). The oxytocin receptor gene is highly expressed in uterine epithelium and peripheral blood, especially during the last parturition and postpartum periods (40). Gonadal steroids play an essential role in regulating the uterine oxytocin receptor gene, especially the plasma ratio of progesterone to estrogen (41).

The current study investigating the expression patterns of the oxytocin receptor gene showed that the oxytocin receptor gene expression was highly expressed in the treated group with exogenous oxytocin compared to the control group injected with normal saline for four weeks. The increase in oxytocin receptor gene expression in the group injected with exogenous oxytocin can be explained. The injection of exogenous oxytocin has a local positive feedback system that causes a continuous increase in prostaglandin synthase and release from the endometrium, leading to autolysis (42). These events caused high upregulation of the oxytocin receptor gene in the endometrium (43). The functional physiology of the oxytocin receptor gene was given by the molecular structure of the receptor and the molecular interaction with other intracellular components (44).

Conclusion

We conclude that injection of exogenous oxytocin at 100 IU/IM twice weekly for four weeks postpartum will decrease the duration of uterine involution and accelerate the occurrence of estrus in the local Iraqi breed of Karradi cows.

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Conflict of interest

No conflict.

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العلاقة بين حقن الاوكسيتوسن وإظهار جين مستقبل الاوكستوسين وتأثيراتهما على أوب الرحم في الأبقار المحلية العراقية

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الخلاصة

هدفت الدراسة الحالية إلى التعرف على تأثير حقن الاوكستوسين الخارجي على فترة أوب الرحم في أبقار الكرادي العراقية المحلية. عشرون بقرة تم تقسيمها إلى مجموعتين، المجموعة الأولى اعتبرت مجموعة سيطرة، أما المجموعة الثانية تم حقنها بمائة وحدة دولية من الاوكسيتوسين مرتين أسبوعياً ولمدة أربعة أسابيع بعد الولادة. تم مراقبة سرعة أوب الرحم من خلال استخدام جهاز فحص الأمواج فوق الصوتية من خلال قياس سمك بطانة الرحم، قطر المبيض، قطر عنق الرحم، وقطر قرن الرحم، فضلا عن تقدير مستوى هرمون الأستروجين والبروجستيرون في مصل الدم باستخدام اختبار الاليزا، تم قياس جميع المعايير السابقة مرتين أسبوعياً ولمدة أربعة أسابيع بعد الولادة. أظهرت نتائج المجموعة المعاملة أن مستوى الأستروجين في مصل الدم ازداد معنوياً بالمقارنة مع مجموعة السيطرة في جميع الفترات، أما سمك بطانة الرحم فقد لوحظ انخفاض سمكها معنوياً عن الأسابيع الثاني والثالث والرابع عند المقارنة مع مجموعة السيطرة، بالإضافة إلى أن قطر المبيض انخفض معنوياً في الأسبوعين الأول والثاني ولكنه ازداد معنوياً في الأسبوع الرابع بالمقارنة مع مجموعة السيطرة، فضلا عن أن قطر عنق الرحم انخفض معنوياً في الأسبوعين الأول والثاني بالمقارنة مع مجموعة السيطرة، وعلى نفس النمط، فان قطر قرن الرحم انخفض معنوياً في جميع الأسابيع عند المقارنة مع مجموعة السيطرة. نستنتج من الدراسة الحالية أن حقن الاوكستوسين الخارجي بجرعة مائة وحدة دولية في العضلة مرتين أسبوعيا ولمدة أربعة أسابيع بعد الولادة يسبب تقليص الفترة الزمنية التي يحتاجها الرحم للعودة إلى الشكل الطبيعي وتسريع عملية أوب الرحم وحدوث دورة شبق جديدة في أبقار الكرادي المحلية العراقية.