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Genetic Expression of Progesterone and Oxytocin Receptors in Kari Sheep during Estrus and Early Pregnancy

Ijaz Ahmad[1], Mohammad Qasim[2], Laiba Saghir[3], Aalam Sher[2], Shahzada Khurram Adrian Shah[4], Sadaf Ilyas[5], Ikramul Haq[6], Azizullah Khan[1], Muhammad Najmus Saqib[4], Shah Faisal[7], Farhad Ali[2]

[1]The University of Agriculture, Peshawar, KP, Pakistan.
[2]Pakistan Agricultural Research Council, Islamabad, Pakistan.
[3]Quaid e Azam University, Islamabad, Pakistan.
[4]University of Veterinary and Animal Sciences, Swat, KP, Pakistan.
[5]University of Sialkot, Punjab, Pakistan.
[6]Livestock & Dairy Development Department, KP, Pakistan.
[7]Abdul Wali Khan University, Mardan, KP, Pakistan.

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Correspondence to: Farhad Ali, Pakistan Agricultural Research Council, Islamabad, Pakistan.
Email: farhad6962@parc.gov.pk

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ABSTRACT

Kari is a small breed of sheep found in Chitral district of Khyber Pakhtunkhwa with unique characteristics having shorter gestation length than other breeds i.e. lesser than five months. In this experiment the expression pattern of oxytocin and progesterone receptors gene in estrus cycle and early pregnancy was studied. For this experiment total seven ewes non-pregnant scanned through ultrasonography and then estrous synchronized with double PGF2α protocol. The fertile rams were allowed, and mating was observed. Blood samples was collected from ewes before and after confirmation of pregnancy. RNA was isolated from the blood and synthesized c-DNA through RT-PCR. The c-DNA was amplified using primers i.e. P4, OXTR and GABDH. No expression of OXTR was observed between 08th and 12th day in control, non-pregnant and pregnant ewes while expression of OXTR was observed on 14th day in non-pregnant and no expression in pregnant ewes. The expression of progesterone receptors was high on 10th and 12th day in pregnant ewes while low level was recorded on 16th day. In control and non-pregnant ewes the expression of P4 mRNA was lower at 08th day and highly expressed on 14th and 16th day of estrus cycle. It is concluded that early pregnancy in ewes sustained by a complex interplay between the conceptus and endometrium. This contact involves factors like IFN-t, progesterone, and growth factors, which regulate gene expression to suppress luteolysis and maintain pregnancy.

INTRODUCTION

Kari is a unique sheep breed found in district Chitral. Kari is a small breed with a thin tailed, having different body color, but 75% breeds found in herd is of white color. The ears of kari sheep are small. Males have strong and thick horned while the females are usually hornless. The average adult body weight is 22 kg for male while for female it is 18 kg. The length of estrus cycle ranges from 16-17 days (*Ahmad et al*., 2002). Length of estrus period can last from 24-36 hours. Normally, ovulation occurs in ewes at end of estrus cycle, usually after 24-27 hours from the period of estrus start. Rate of ovulation increases with increase an age of animal, reached to peak at 6 years of age, and then gradual decline occur. Eggs are released after ovulation, having the capability for 10-25 hours for fertilization. (Gimenez and Rodning, 2007). In Kari sheep gestation period ranges from 3-5 months usually less than 5 months. Birth weight of lamb born single is 2.7 kg which is 24% heavier than the ones born in twin. Weight of lamb at weaning is 3.63 kg for single born while for twin born it is 3.23 kg. Kari sheep produce 4 Iambs per annum (Ahmad *et a.,l* 2002).

The estrus cycle of mammalian species consists of follicular and luteal phases, controlled by various reproductive organs and hormones released from different parts of the body (Soede *et al.,* 2011). An absence of embryo in uterus, PGF2α causes luteolysis which result a rapid decrease in blood progesterone levels. The secretion of PGF2α pulses essential for luteolysis in sheep, reliant on the effects of progesterone, estrogen and oxytocin in the uterus (Thatcher *et al.,* 1995, Bazer *et al.,* 1994). Progesterone acts on uterus to increase production of phospholipid and activity of cyclooxygenase needed for renovation of archidonic acid to PGF2α.

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The maternal system prevents luteolysis after fertilization by trophoblast and maintains pregnancy through production of progesterone essential for pregnancy development (Geisert and Malayer. 2000). The o-IFN-τ is secreted between day 10 and 21 of pregnancy acts locally on the uterus, having different properties i.e. anti-luteolytic, anti-viral, anti-proliferative and immunosuppressive properties (Jainudeen and Hafez, 2000, Bazer *et al.,* 1989). In ewes, IFN-τ persuades a potent suppressor (IRF2), which calms transcription of estrogen receptors. In the lack of estrogen receptors, estrogen is incapable to induce expression of oxytocin receptors in uterine epithelia results in termination of oxytocin secretion (Bazer *et al*., 2011).

Therefore, the current study aims to investigate the regulation of progesterone and oxytocin receptors during estrus cycle and early pregnancy in ewes and to determine the effects of oxytocin receptor in detection of early pregnancy in ewes.

MATERIALS AND METHODS

Animal Selection and estrous synchronization

A total of 07 non-pregnant, sexually matured ewes having average body weight of 16 kg, being scanned b ultrasound. All the ewes synchronized using double PGF2α protocol and then fertile rams were allowed to ensure mating with ewes.

Blood Samplings and RNA Isolation

Seven ml of blood sample was collected from each non-pregnant ewe on day 8th, 10th, 12th, 14th and 16th of estrus cycle once daily at morning 8:00 to 9:00 am in EDTA tubes. RNA was isolated form samples by TRIZOL reagents. The concentrations of total RNA were determined by measurement of optical density at 260/280 nm in the UV spectrophotometer (Beckmann).

c-DNA Synthesis

c-DNA synthesis kit (Thermo scientific) was used to synthesize c-DNA from mRNA according to manufacturer's protocol using RT-PCR i.e. by mixing Primer oligo dt 1µl, 5x reaction buffer 4µl, RNase inhibitor 1µl, DNTPs 0.4µl, Reverse aid enzyme 1µl, ddH2O 2.6µl and RNA sample 10µl. The condition for RT-PCR was extension for 59 minutes at 42^{0}C, termination for 5 minutes at 70^{0}C and holding for 2 minutes at 22^{0}C.

PCR Amplification

The quantitative PCR was performed using Dream-Taq Green PCR Master Mix (2x) (Thermo scientific) for a total of 35 cycle by using the following PCR mixture and thermo cycling condition c-DNA 1 ul, Master mix 5 ul, Primers1 ul (each *P4, OXTR* and *GABDH*), dH20 3 ul with total reaction of 10 ul. The conditions for amplification of *P4, OXTR* and *GABDH* was Initial denaturation at 94^{0}C for 5 minutes 1x, denaturation at 94^{0}C for 30 seconds, Primer annealing at 58.1^{0}C for 30 seconds 35x, extension at 72^{0}C for 40 seconds and final extension at 72^{0}C for 10 minutes.

Gel Electrophoresis

The PCR amplicons was detected in 1% agarose gel electrophoresis medium using florescent dye and electric supply.

Data Analysis

Data were analyzed through image j software version 1.47 and graph pad prism 6 software.

RESULTS AND DISCUSSION

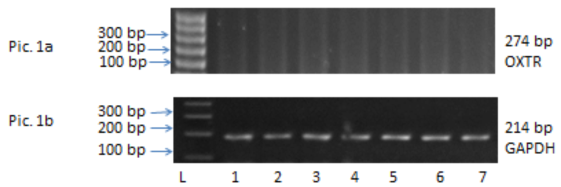
Expression of OXTR in control, non-pregnant and pregnant ewes

No expression of *OXTR* was observed between day 8th and 12th of estrus cycle in control and non- pregnant ewes while expression was observed on day 14th and reached to peak level on day 16th. In pregnant ewes *OXTR* showed a similar pattern of expression between day 8th and 12th but they differ on day 14th and 16th as no receptors were expressed. Moreover, the expression of GABDH was observed in all the stages showing the validation of experiment.

This result is consistent with the results of Wathes and Hamon 1993. They conducted a study on uterine tissue collected from 47 ewes to regulate the localization of receptors for steroid and oxytocin using monoclonal antibodies and concluded that *OXTR* were not detectable between day 5th and 12th of estrus cycle and first developed in luminal epithelium on day 14th of estrus cycle and then reached to peak level in myometrium at estrus in cyclic ewes. In pregnant ewes particular binding site was not visible on day 14 -21.

Figure 1

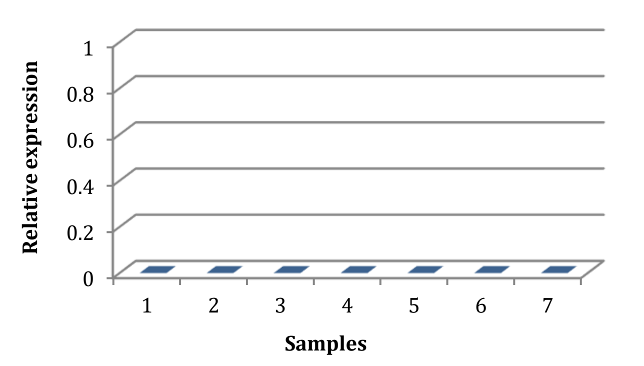
Expression of oxytocin receptor on day 8th of estrus cycle



Gel Picture 1a,2b shows the expression of OXTR and GAPDH gene in control pregnant and non pregnant ewes on day 8 respectively. Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 1

Relative expression of oxytocin receptor on day 8th of estrus cycle

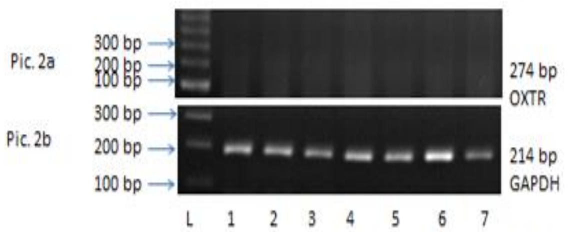


Graph 1 illustrates the relative expression of *OXTR* on day 8th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *OXTR* gene while the x-axis shows the number of ewes.

Figure 2

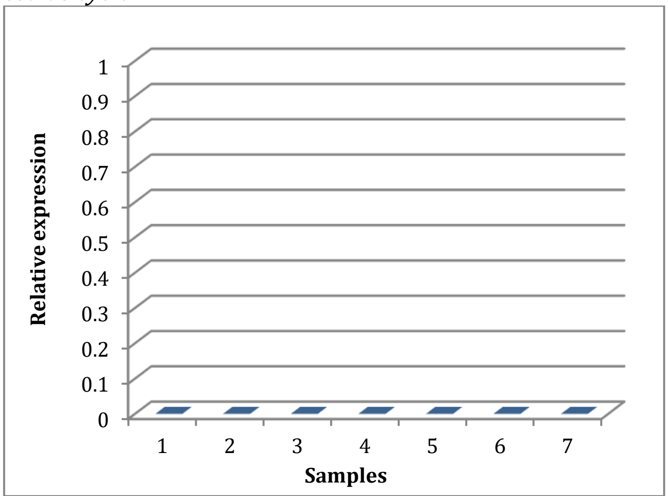
Expression of oxytocin receptor on day 10th of estrus cycle



Gel Picture 2a,2b shows the expression of OXTR and GAPDH gene in control pregnant and non pregnant ewes on day 10 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 2

Relative expressions of oxytocin receptor on day 10th of estrus cycle

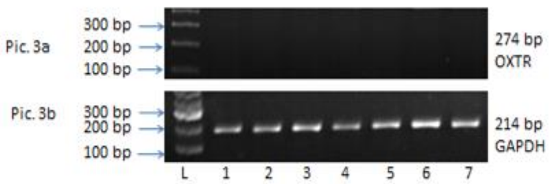


Graph 02 illustrates the relative expression of *OXTR* on day 10th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *OXTR* while the x-axis shows the number of ewes.

Figure 3

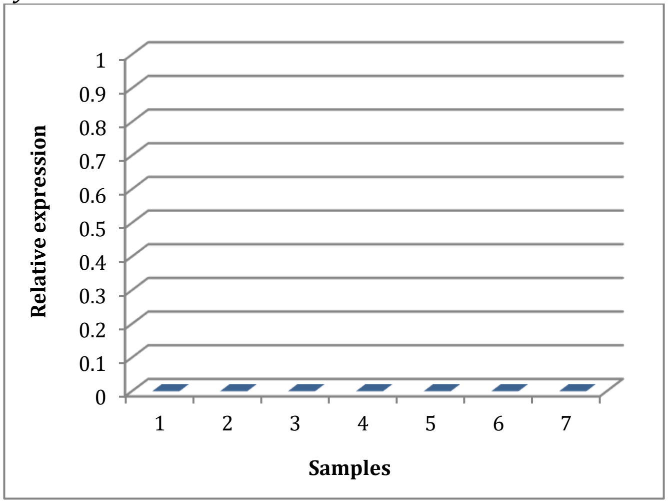
Expression of oxytocin receptor on day 12th of estrus cycle



Gel Picture 3a,3b shows the expression of OXTR and GAPDH gene in control pregnant and non pregnant ewes on day 12 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 3

Relative expression of oxytocin receptor on day 12th of estrus cycle

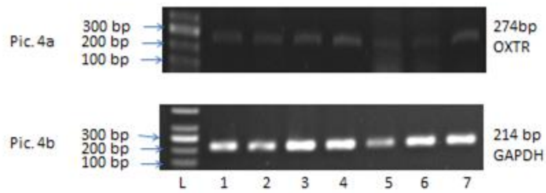


Graph 3 illustrates the relative expression of *OXTR* on day 12th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *OXTR* while the x-axis shows the number of ewes.

Figure 4

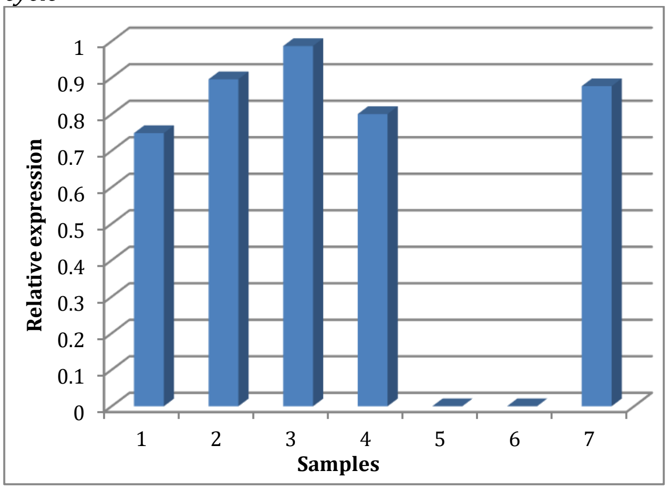
Expression of oxytocin receptor on day 14th of estrus cycle



Gel Picture 4a,4b shows the expression of OXTR and GAPDH gene in control, pregnant and non pregnant ewes on day 14 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 4

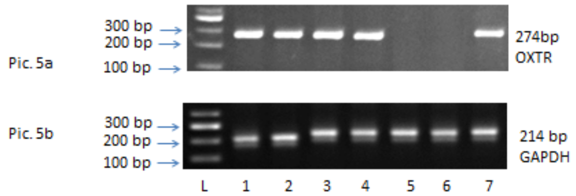
Relative expression of oxytocin receptor on day 14th of estrus cycle



Graph 4 illustrates the relative expression of *OXTR* on day 14th in control, pregnant and non-pregnant ewes.
The y-axis indicates the relative expression of *OXTR* while the x-axis shows the number of ewes.

Figure 5

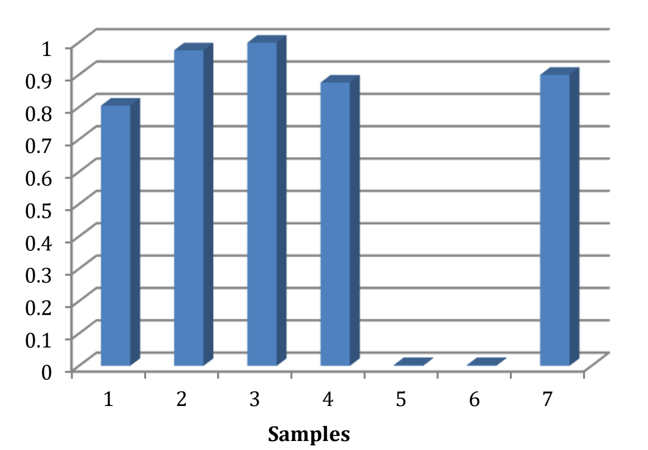
Expression of oxytocin receptor on day 16th of estrus cycle



Gel Picture 5a,5b shows the expression of OXTR and GAPDH gene in control pregnant and non pregnant ewes on day 16 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 5

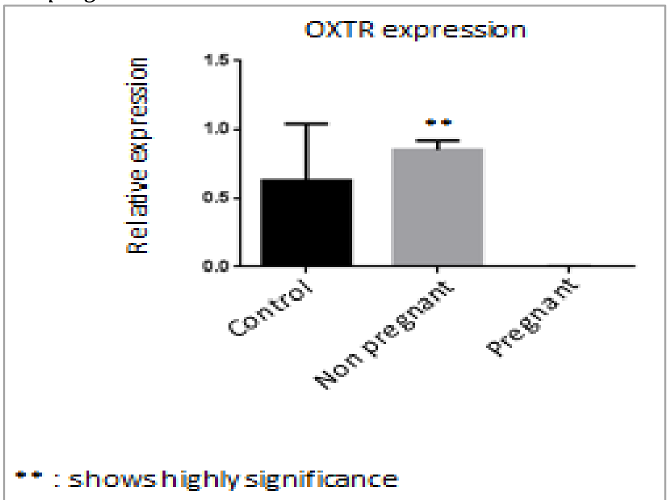
Relative expression of oxytocin receptor on day 16th of estrus cycle



Graph 5 illustrates the relative expression of *OXTR* on day 16th in control, pregnant and non-pregnant ewes.The y-axis indicates the relative expression of *OXTR* while the x-axis shows the number of ewes.

Graph 6

Expression of oxytocin receptor in control, non pregnant and pregnant ewes



Graph 06 illustrates the relative expression of *OXTR* in control, pregnant and non pregnant ewes. The y-axis indicates the relative expression of *OXTR* while the x-axis shows the groups of ewes.

Results from the analysis of variance for *OXTR* shows that there is highly significant difference found in *OXTR* concentrations between pregnant and non-pregnant ewes ($P<0.01$). In control and non-pregnant ewes there is little statistical difference ($P<0.05$).

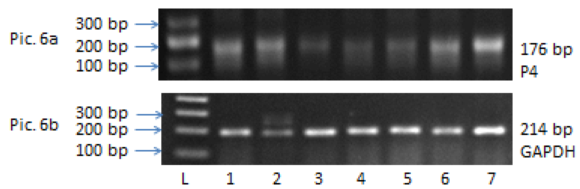
Expression of *P4* in control, pregnant and non-pregnant ewes

Expression of P4 was observed in all the three groups of ewes (control, non-pregnant and pregnant) but the greatest amount was recorded at days 10th and 12th in pregnant ewes while low level was recorded on day 16th of estrus cycle. In control and non-pregnant ewes, the amount of progesterone receptor mRNA was lowest at day 08th and increased to days 14th and 16th. Moreover, the expression of GABDH was observed in all the stages showing the validation of experiment.

The findings of this study are in line with Ott *et al* 1993, a study on mature crossbred Rambouillet ewes. Ewes were naturally inseminated and then hysterectomized at different days of estrus cycle (10, 12, 14, or 16). Endometrium was collected and stored for further processing. Furthermore, their results showed that in non-pregnant ewes, the expression of progesterone receptors mRNA improved from day 10th to a peak level on days 14th and 16th of estrus cycle. In pregnant ewes, progesterone receptors mRNA levels were highest on day 10th and 12th and decline almost 50% by day 16th of cycle.

Figure 6

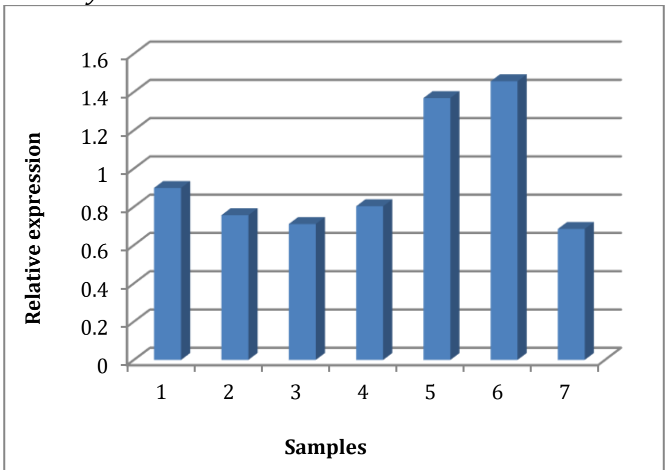
Expression of progesterone receptor on day 8th of estrus cycle



Gel Picture 6a,6b shows the expression of P4 and GAPDH gene in control pregnant and non pregnant ewes on day 08 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 7

Relative expression of progesterone receptor on day 8th of estrus cycle

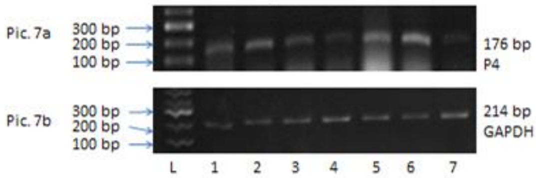


Graph 7 illustrates the relative expression of *P4* on day 8th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the number of ewes.

Figure 7

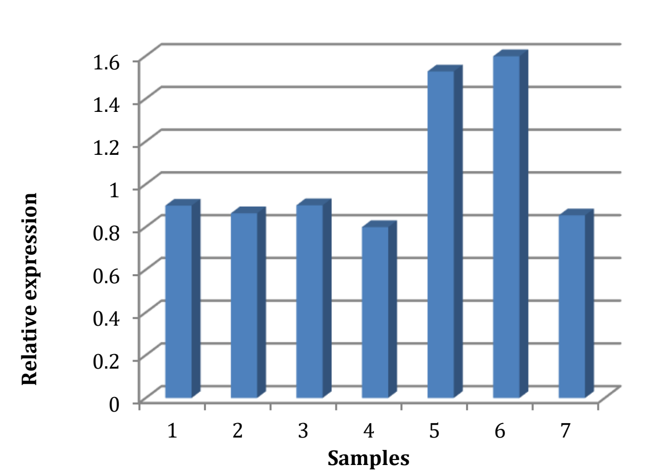
Expression of progesterone receptor on day 10th of estrus cycle



Gel Picture 7a,7b shows the expression of P4 and GAPDH gene in control pregnant and non pregnant ewes on day 10 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 8

Relative expression of progesterone receptor on day 10th of estrus cycle

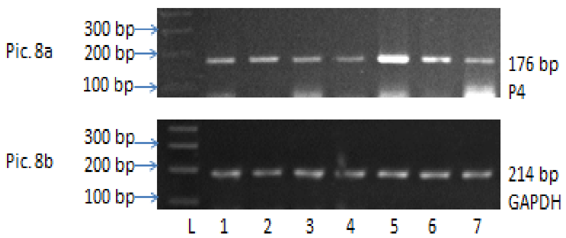


Graph 8 illustrates the relative expression of *P4* on day 10th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the number of ewes.

Figure 8

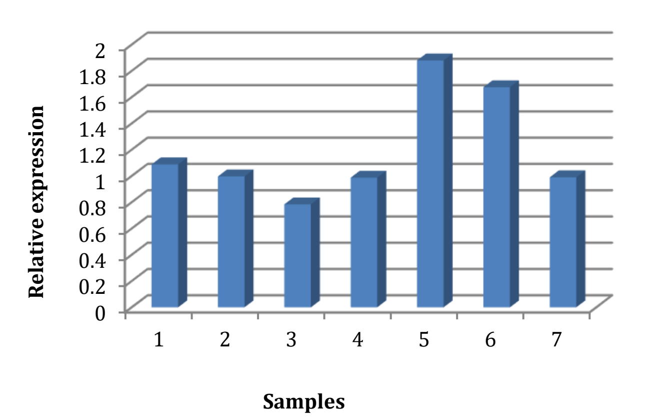
Expression of progesterone receptor on day 12th of estrus cycle



Gel Picture 8a,8b shows the expression of P4 and GAPDH gene in control pregnant and non pregnant ewes on day 12 respectively. Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 9

Relative expression of progesterone receptor on day 12th of estrus cycle

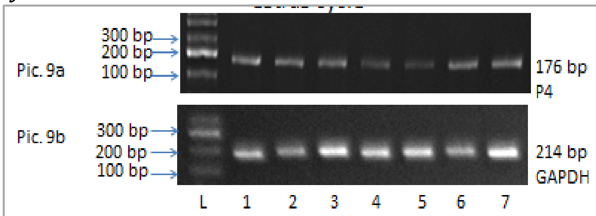


Graph 9 illustrates the relative expression of *P4* on day 12th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the number of ewes.

Figure 9

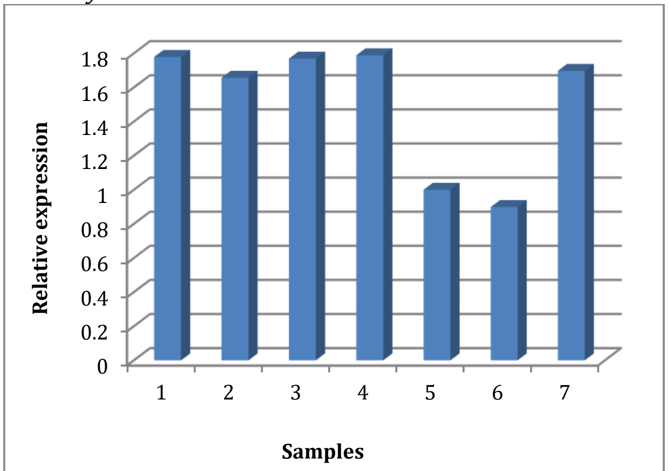
Expression of progesterone receptor on day 14th of estrus cycle



Gel Picture 9a,9b shows the expression of P4 and GAPDH gene in control pregnant and non pregnant ewes on day 14 respectively.
Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 10

Relative expression of progesterone receptor on day 14th of estrus cycle

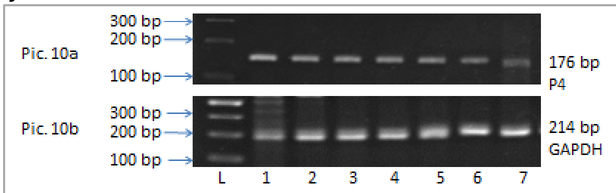


Graph 10 illustrates the relative expression of *P4* on day 14th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the number of ewes.

Figure 10

Expression of progesterone receptor on day 16th of estrus cycle

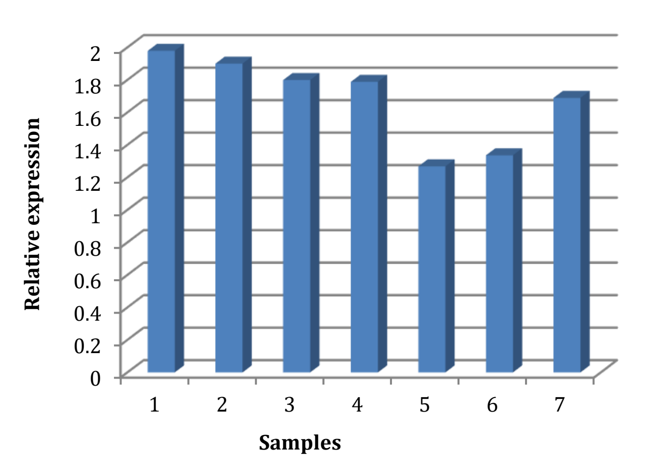


Gel Picture 10a,10b shows the expression of P4 and GAPDH gene in control pregnant and non pregnant ewes on day 16 respectively.

Lane L:100 bp DNA ladder, L: 3, 7. Control group. L: 5, 6. Pregnant sheep. L: 1, 2, 4. non pregnant sheep

Graph 11

Relative expression of progesterone receptor on day 16th of estrus cycle

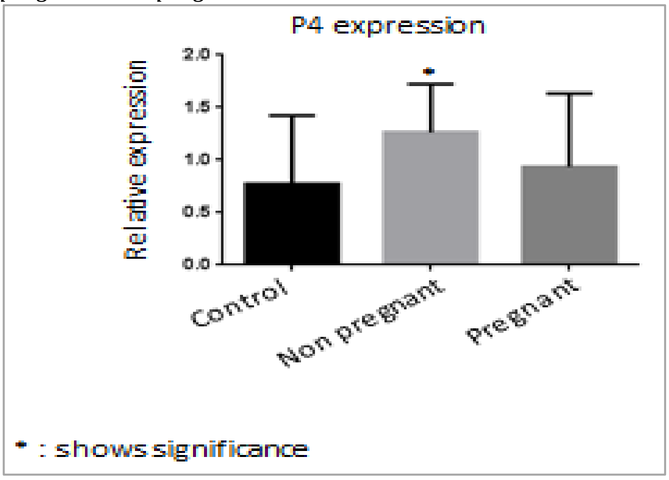


Graph 11 illustrates the relative expression of *P4* on day 16th in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the number of ewes.

Graph 12

Expression of progesterone receptor in control, non-pregnant and pregnant ewes



This graph illustrates the relative expression of *P4* in control, pregnant and non-pregnant ewes.

The y-axis indicates the relative expression of *P4* while the x-axis shows the groups of ewes. Results from the analysis of variance for progesterone receptor mRNA shows that there are little changes found in all groups. In non-pregnant group value is high ($P<0.05$) as compared to control and pregnant group.

CONCLUSION

From the findings of current study, it was concluded that in pregnant ewes, OXTR expression is suppressed, and progesterone expression was at peak around day 8th to 10th. While non-pregnant ewes show increased OXTR on day 14 and peak progesterone receptors on days 14-16. Furthermore, it was observed that conceptus-endometrium interactions, involving IFN-τ, progesterone, and growth factors, regulate gene expression and maintain pregnancy.

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