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Relative expression of oxytocin receptor gene in buffalo endometrium in late luteal phase and pregnancy stages

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ABSTRACT

Molecular level information related to buffalo (*Bubalus bubalis*) reproduction and related genes is not present at appropriate level. If such exploration is made in the form of comparison between expression of genes is made between non-pregnant and pregnant phase, it may be helpful to aid manipulate the reproduction. Hence, the present study was carried out to reveal mRNA quantitative real time expression of oxytocin receptor (OTR) mRNA. IFN-τ is considered as the substance of maternal recognition of pregnancy and shut down the probable mechanisms which lead to luteolysis. Such mechanism includes shutting down of OTR. Therefore, relative expression of OTR was studied in endometrial tissue of three groups. The groups were non-pregnant late luteal phase, pregnancy stage I (pregnancy of <42 days) and pregnancy stage II (>42 days of pregnancy). With designed primer and GAPDH as house-keeping gene, relative mRNA expression was measured in Real-time PCR. After statistical analysis of results, the gene found to be expressed in all three stages with non-significant difference.

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KEYWORDS
Buffalo; IFN-τ; oxytocin receptor; pregnancy; endometrium; relative expression

Introduction

In Bovines, 60–70% of pregnancy losses take place in first three weeks of the pregnancy, the period of pregnancy recognition (Sreenan & Diskin 1983). Simultaneously, many factors have been found to be involved in pregnancy success, viz communication established between embryonic and maternal system, cessation of luteal regression and responsible mechanisms, the immune response favourable for pregnancy and preparation of the uterus for implantation (Wang & Dey 2006). Failure of manner of coordination between mechanisms behind the phenomenon of pregnancy even may lead to termination of pregnancy. In cattle, corpus luteum (CL) of pregnancy produces more Prostaglandin E_2 (PGE_2) (Weems et al. 1998) and less oxytocin (OT) (Wathes et al. 1984) than the CL of the cycle. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a luteolysin in buffalo as in cattle (Skarzynski et al. 2003) and sheep (Jenkin 1991), is responsible for luteal regression and commencement of next oestrous cycle (McCracken et al. 1984) which is opposite to pregnancy. During luteal regression, episodic pulses of OT secretion become united to the release of $PGF_{2\alpha}$ following synthesis of endometrial OT receptors (OTRs). When pregnancy get established, a signal is sent by conceptus itself in form of interferon-τ (IFN-τ) which ensures maintenance of CL and continued progesterone (P4) production that is must for sustaining pregnancy (Sakumoto et al. 2014). In both sheep and cattle, IFN-τ prevents up-regulation of OTR expression in the endometrial luminal epithelium and superficial glandular epithelium (Dorniak et al. 2012) as well as development of the pulsatile pattern of $PGF_{2\alpha}$ release needed to attain luteolysis (Wathes & Lamming 1994). It can be seen that in order to prevent embryonic loss and to withstand the pregnancy, the comparative expression of single gene may contribute which is also found in case of expression of OTR. Hence, the importance of relative expression of OTR is noticeable during oestrous cycle and pregnancy. By keeping all these facts, the present study was directed to study relative expression of OTR in endometrial tissue of non-pregnant cyclic (late luteal phase) and pregnant animals. Preceding to the follicular phase in cyclic animals, late luteal phase lies, which is common in pregnant and cyclic animals. While in pregnancy stages, two different stages were chosen namely, pregnancy stage I (<42 days of pregnancy) and pregnancy stage II (>42 days of pregnancy). The intension was to reveal the relative expression of OTR between three chosen physiological conditions of reproduction in buffalo.

Material and methods

Experimental animals

Apparently healthy female buffalo reproductive tracts ($n = 6$ for each group) were procured from the abattoir located in Bareilly, Uttar Pradesh, India immediately after slaughter under aseptic conditions and transported to laboratory on ice. External and internal characteristics of the CL, endometrium and cervix were examined (Arosh et al. 2002) for non-pregnant late

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luteal stage. For predicting the stage of pregnancy, pregnant samples were brought and processed immediately. The foetus with foetal membrane was carefully removed and on the basis of crown rump/crown vertebral rump length, approximately days of pregnancy was decided (Assis Neto et al. 2010).

Collection of tissue samples

Uteri were dissected from the surrounding tissues, washed with DEPC-treated phosphate buffered saline, and cut open on their longitudinal axis along the greater curvature. Approximately 100 mg endometrium tissue was collected in 1 ml of RNAlater and kept at −20°C until use.

RNA isolation and first strand cDNA synthesis

Total RNA isolated from all stored endometrial samples with QIAGEN RNeasy plus mini kit followed by checking the quality and concentration of isolated RNA by spectrophotometric and electrophoretic analysis. After DNase treatment (Fermentas), approximately 2 µg of RNA was reverse transcribed to first strand cDNA by high-capacity RNA-to-cDNA kit (Applied Biosystems by Life Technologies, USA).

Primer designing and standardization

Gene specific pairs of primers were designed for OTR and GAPDH gene from IDT. The detail of primers is given in Table 1. Prepared cDNA was then standardized to decide annealing temperature (Table 1) with PCR followed by checking of amplified product on 2% gel electrophoresis.

Real-time PCR

The expression study was carried out by quantitative Real-time PCR (qRT-PCR) (Step One Plus by Applied Biosystems®, USA) by using standardized gene specific primers (Table 1) in triplicate. The amplification was carried out in 10 µL reaction volume using Fast SYBR Green qPCR Master mix and following thermal profile of the same. Negative and positive controls were included for the qPCR assay. For each sample, a dissociation curve was generated after completion of amplification and analysed in comparison to negative and positive controls, to determine specificity of PCR reaction.

Quantification of gene expression and statistical analysis

C_T values of gene were normalized with that of GAPDH to obtain ΔC_T followed by calculation of $\Delta\Delta C_T$ by keeping non-pregnant late luteal stage as calibrator. Fold change was calculated (Livak & Schmittgen 2001) and Log_2 fold change was presented graphically. GraphPad PRISM v.5.0 software (GraphPad Software, Inc., San Diego, CA) was used to perform the statistical analysis. Kruskal–Wallis non parametric test was performed to find the significant differences between ΔC_Ts of groups. The confidence level was set at 95%. Dunn's multiple comparison test was chosen for the pairwise comparisons. Significance threshold was set at p-value <.05.

Results

Sample collection

Total 18 samples, $n = 6$ from each group were collected. In pregnancy stages, samples from pregnancy stage I were in range of 28–38 days and pregnancy stage II samples were from 48–56 days of pregnancy which was decided on the basis of foetal crown rump/crown vertebral rump length measurement.

Relative expression of OTR mRNA in endometrium during oestrous cycle and pregnancy

The amplification plot of targeted region and their dissociation curve is given in Figures 1 and 2. A single dissociation curve peak indicates absence of any non-specific amplification at annealing temperature used in analysis. qRT-PCR analysis did not reveal significant differences in OTR gene expression in buffalo endometrium between non-pregnant stage, pregnant stage I and pregnant stage II. Relative expression of targeted gene between selected groups ($p > .05$) is shown in Figure 3. The gene has been found up-regulated in pregnant stages but with non-significant difference.

Discussion

During oestrous cycle and among different phases, the OTR mRNA expression is found highest prior and a few days after the luteolysis, that is, around 17 days of previous cycle up to 5 days of the next cycle (Jenner et al. 1991; Robinson et al. 2001). During rest of the oestrous cycle, it remains at a comparatively lower level and even undetectable between days 10 and 15 in the epithelium (Robinson et al. 2001). The stage taken in this study as a control for comparison is the late luteal phase of oestrous cycle, that is, from 11 to 16 days of oestrous cycle and is the period in which OTR are at the lowest concentration. OT creates its effect positively on $PGF_{2\alpha}$ and its pulsatile release suggesting its role in luteolysis (Spencer & Bazer 1996). The mRNA expression has been found down regulated in the window of maternal recognition of pregnancy in various species *in vivo* and *in vitro* (Salamonsen & Findlay 1990; Raw et al. 1995; Sharp et al. 1997; Kimmins & MacLaren 2001; Bazer et al. 2008). At the same time, in bovine endometrial

Table 1. Sequence of designed primers of OTR and GAPDH.

Gene	Primer sequence	Amplicon length	Annealing temperature
OTR	F: 5′-CCTGGATCTACATGCTCTTCAC-3′ R: 5′-GGTGGACGAGTTGCTCTTT-3′	134 bp	60°C
GAPDH	F: 5′-TGACCCCTTCATTGACCTTC-3′ R: 5′-GATCTCGCTCCTGGAAGATG-3′	143 bp	60°C

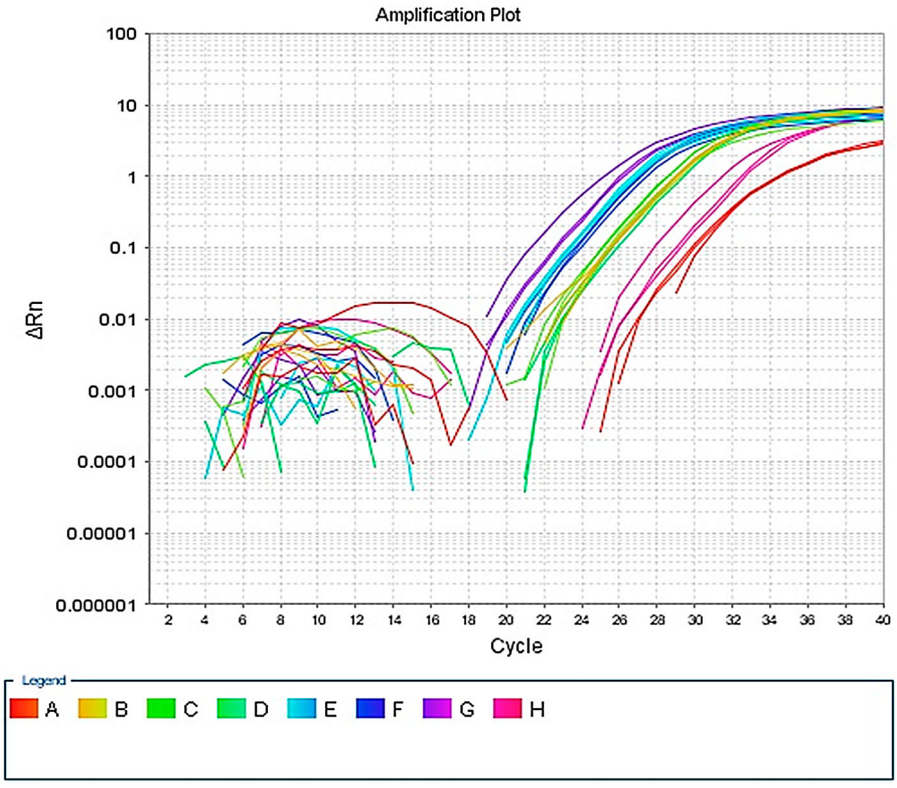

Figure 1. Amplification plot of OTR gene (log view).

epithelial cell line, it has been also demonstrated that OT under the effect of IFN-τ, suppressed $PGF_{2\alpha}$ accumulation without modulating OTR expression (Krishnaswamy et al. 2009) suggesting the presence of alternative or supportive action of OT on the $PGF_{2\alpha}$ and luteolysis along with mediation through OTR. In ewes, it has been hypothesized that the inhibitory action of IFN-τ on OTR up-regulation is achieved indirectly by inhibiting the up-regulation of oestrogen receptor α (Lamming et al. 1995, Spencer et al. 1995).

In the current study, the samples from early pregnancy are ranging approximately from 28 to 38 days, which includes post-maternal recognition stage along with lowered IFN-τ concentration. This may be the reason for not getting the significant OTR down-regulation. Simultaneously, being hormone of pregnancy, P4 remains elevated in pregnancy. This may have effects on the conformation of receptor as illustrated in one

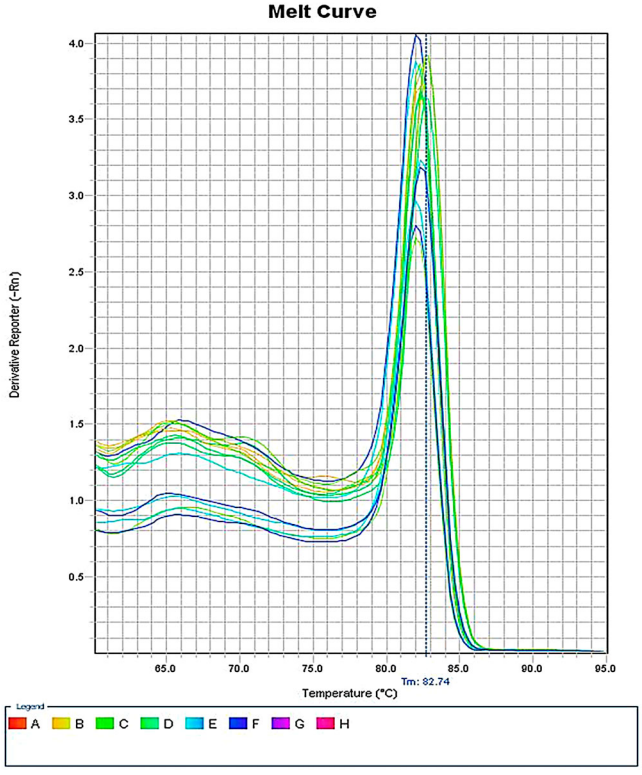

Figure 2. Dissociation curve of OTR gene.

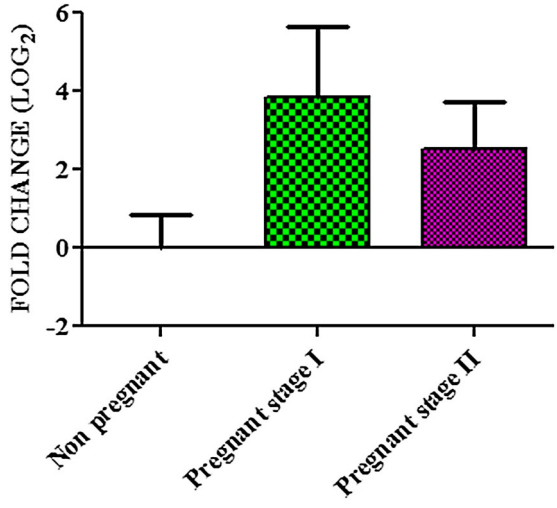

Figure 3. Relative mRNA expression of OTR gene of different groups.

study that P4 can influence the cholesterol in cell membrane and resulting in conformational changes in OTR, resulting inhibition of OT action (Gimpl & Fahrenholz 2002). In conclusion, the results of OTR relative expression showed non-significant difference which is different than that of earlier reported in bovines. It may suggest the presence of alternative or supportive action of OT on luteolysis along with OTR.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Arosh JA, Parent J, Chapdelaine P, Sirois J, Fortier MA. 2002. Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. Biol Reprod. 67:161–169.

Assis Neto AC, Pereira FT, Santos TC, Ambrosio CE, Leiser R, Miglino MA. 2010. Morpho-physical recording of bovine conceptus (Bos indicus) and placenta from days 20 to 70 of pregnancy. Reprod Domest Anim. 45:760–772.

Bazer FW, Burghardt RC, Johnson GA, Spencer TE, Wu G. 2008. Interferons and progesterone for establishment and maintenance of pregnancy: interactions among novel cell signaling pathways. Reprod Biol. 8:179–211.

Dorniak P, Bazer FW, Wu G, Spencer TE. 2012. Conceptus-derived prostaglandins regulate endometrial function in sheep. Biol Reprod. 87 (1):9–9.

Gimpl G, Fahrenholz F. 2002. Cholesterol as stabilizer of the oxytocin receptor. BBA-Biomembranes. 1564:384–392.

Jenkin G. 1991. Oxytocin and prostaglandin interactions in pregnancy and at parturition. J Reprod Fertil. 45:97–111.

Jenner LJ, Parkinson TJ, Lamming GE. 1991. Uterine oxytocin receptors in cyclic and pregnant cows. J Reprod Fertil. 91:49–58.

Kimmins S, MacLaren LA. 2001. Oestrous cycle and pregnancy effects on the distribution of oestrogen and progesterone receptors in bovine endometrium. Placenta. 22(8):742–748.

Krishnaswamy N, Danyod G, Chapdelaine P, Fortier MA. 2009. Oxytocin receptor down-regulation is not necessary for reducing oxytocin-induced prostaglandin F2α accumulation by interferon-τ in a bovine endometrial epithelial cell line. Endocrinology. 150:897–905.

Lamming GE, Wathes DC, Flint APF, Payne JH, Stevenson KR, Vallet JL. 1995. Local action of trophoblast interferons in suppression of the development of oxytocin and oestradiol receptors in ovine endometrium. J Reprod Fertil. 105:165–175.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 25:402–408.

McCracken JA, Schramm W, Okulicz WC. 1984. Hormone receptor control of pulsatile secretion of PGF2α from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim Reprod Sci. 7:31–55.

Raw RE, Silvia WJ, Curry TE. 1995. Effects of progesterone and estradiol on prostaglandin endoperoxide synthase in ovine endometrial tissue. Anim Reprod Sci. 40:17–30.

Robinson RS, Mann GE, Lamming GE, Wathes DC. 2001. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. Reproduction. 122:965–979.

Sakumoto R, Hayashi KG, Takahashi T. 2014. Different expression of PGE synthase, PGF receptor, TNF, Fas and oxytocin in the bovine corpus luteum of the estrous cycle and pregnancy. Reprod Biol. 14:115–121.

Salamonsen LA, Findlay JK. 1990. Immunocytochemical localization of prostaglandin synthase in the ovine uterus during the oestrous cycle and in early pregnancy. Reprod Fertil Develop. 2:311–319.

Sharp DC, Thatcher MJ, Salute ME, Fuchs AR. 1997. Relationship between endometrial oxytocin receptors and oxytocin-induced prostaglandin F2α release during the oestrous cycle and early pregnancy in pony mares. J Reprod Fertil. 109:137–144.

Skarzynski DJ, Jaroszewski JJ, Bah MM, Deptula KM, Barszczewska B, Gawronska B, Hansel W. 2003. Administration of a nitric oxide synthase inhibitor counteracts prostaglandin F2-induced luteolysis in cattle. Biol Reprod. 68:1674–1681.

Spencer TE, Bazer FW. 1996. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. Endocrinology. 137:1144–1147.

Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, Bazer FW. 1995. Ovine interferon-tau inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinology. 136:4932–4944.

Sreenan JM, Diskin MG. 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet Rec. 112:517–521.

Wang H, Dey SK. 2006. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet. 7:185–199.

Wathes DC, Lamming GE. 1994. The oxytocin receptor, luteolysis and the maintenance of pregnancy. J Reprod Fertil. 49:53–67.

Wathes DC, Swann RW, Pickering BT. 1984. Variations in oxytocin, vasopressin and neurophysin concentrations in the bovine ovary during the oestrous cycle and pregnancy. J Reprod Fertil. 71:551–557.

Weems YS, Lammoglia MA, Vera-Avila HR, Randel RD, Sasser RG, Weems CW. 1998. Effects of luteinizing hormone (LH), PGE 2, 8-Epi-PGE 1, 8-Epi-PGF 2 α, trichosanthin and pregnancy specific protein B (PSPB) on secretion of prostaglandin (PG) E (PGE) or F 2 α (PGF 2 α) in vitro by corpora lutea (CL) from nonpregnant and pregnant cows. Prostag Oth Lipid Me. 55:359–376.