Promotor Analysis of cattle endometrium throughout oestrous cycle and early gestation period

Abstract

Endometrial gene expression is primarily regulated by the ovarian steroids and pregnancy recognition factors. A significant number of analyses of differential expression genes (DEGs) in bovine endometrium have been reported. Bovine uteri at *follicular stage (FS), luteal stage (LS) and implantation stage (IS).*Promoter, a region of DNA sequences, defines where transcription of a gene begins by RNA polymerase. promoter sequences are naturally located directly upstream of the transcription start site. RNA polymerase and the basic transcription factors (TFs) bind to the promoter sequence and start transcription. TFs,belong to growing family of regulatory protein, influence transcription by regulating many different cellular function by interacting directly with DNA.1kb upstream of the promoter region of each DEGs analyzed by NSITE (Recognition of Regulatory motifs) of Softberry (http://www.softberry.com) and predicated several TFs binding site. GO (gene ontology) used for identification of those DEGs have TFs functions. Promoter analyses estimated 150-160 TFs for each stage. DLX4 and IRF4 at FS, and IRF5, IRF9, STAT1 and STAT2 at IS were in common to DEGs and estimated TFs, respectively.The present study highlighted potential molecular mechanisms controlling endometrial function during estrus cycle and implantation stage, which will further guide to better understand the endometrial functions in future studies.

Keyword: endometrium, bovine, transcription factor and promotor

Introduction

Promoter, a region of DNA sequences, defines where transcription of a gene begins by RNA polymerase. promoter sequences are naturally located directly upstream of the transcription start site.

RNA polymerase and the basic transcription factors (TFs) bind to the promoter sequence and start transcription (Pedersen*et al.* 1999). TFs,belong to growing family of regulatory protein, influencetranscription by regulating many different cellular function by interacting directly with DNA (Fulton*et al.*2009). TFs can control when, where and how RNA polymerases start. TFs cause increasing or decreasing of gene transcription, protein synthesis, and subsequently changing cellular function.

Many TFs have been recognized and a large proportion of the human genome appears to code for these proteins. Several families of TFs exist and members of each family may share structural characteristics (Whitmarsh and Davis.2000). Some TFs are common in several cell

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types (ubiquitous), they play a general role in the regulation of inflammatory genes, whereas others are cell-specific and may determine the phenotypic characteristics of a cell.

Several report have been done to investigate mammalian TFs at high resolution and depth. Many studies have inferred TF expression through mRNA expression profiling using RNA sequencing (RNA-seq) combined with genome promoter analysis in human (Lambert *et al.*2009).

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But in bovine endometrium promoter analysis during estrous cycle and implantation, it has not been reported yet and in the present study,we were analysed 1kb promoter region of upstream of each DEG genes in bovine endometrium to compare FS vs. LS and LS vs. IS.***The specific aims of this study are to analysis the promoter region of DEGs obtained after comparing each stage.***

Material and methods

Predication of TFs binding site

1kb upstream of the promoter region of each DEGs analyzed by NSITE (Recognition of Regulatory motifs) of Softberry (http://www.softberry.com) and predicated several TFs binding site (Solovyev*et al.* 2010).

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Identification the specific and common TFs

The TFs predicated at each comparing groups in bovine endometrium were include specific and common TFs. The specific transcription factors related to individual one stage and common transcription factors involved in both comparing stage. Subsequently the common and specific TFs were separated.

Identification of TFs from DEGs

The TFs estimated at bovine endometrium have need confirmation, for confirmation the estimated TFs compared with DEGs TFs. GO (gene ontology) used for identification of those DEGs have TFs functions.

Results

Estimated TFs binding site

In the present study, 1 Kb of the upstream region of the promoter of each DEGs were analyzed to predict transcription factor-binding site. While comparing at FS and LS, a total of 153 and 156 TFs were estimated for highly expressed DEGs at FS and LS, respectively.*Similarly, when comparing LS and IS, 157 and 153 TFs were estimated for higher and lower expressed DEGs at IS, respectively.*

Identification the specific and common TFs

When the total number of TFs estimated then separated the common and specific TFs, 26 were specific for DEGs expressed higher at FS and 29 were specific for higher at LS, respectively.

There were 127 estimated TFs in common at FS and LS. Specific TFs were 33 and 29for higher and lower expressed DEGs at IS, respectively, there were 124 TFs estimated in common at IS and LS (Fig. 1).

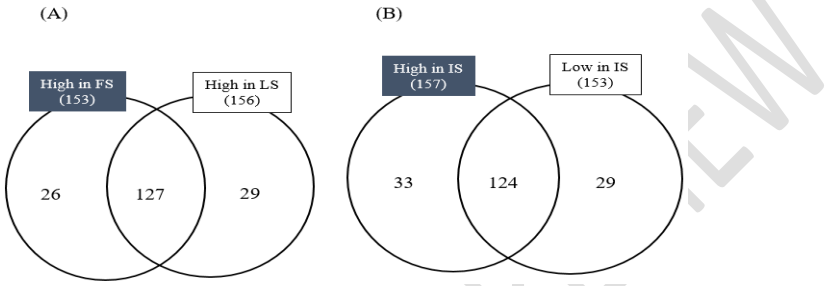

Fig. 1. Venn diagram showing the number of TFs estimated from the promoter region of DEGs at FS vs. LS (A), and LS vs. IS (B). Numbers in the parenthesis indicate the total number of estimated TFs for each stage.

While evaluating from the number of DEGs with binding regions, top five TFs were estimated while comparing at different stages in bovine endometrium are shown in Table 1.

In case of FS vs. LS, the number of DEGs with binding regions, have the large number of DEGs was estimated. For FS: *RFX, RARA, GRDBD, RZR/ROR and MYF*. For LS the five top TFs estimated they bind with large number of DEGs they are:*SP1, PRKCA, SP3, SP2 and IRF1*.

In case of IS vs. LS for IS:SP1, IRF9, IRF5, PRKCA and ZIC1 transcription factors predicated they have the large numbers of DEGs. SP1, SP3, PRKCA, AP2 and HR transcription factors expected they have the large numbers of DEGs with the binding site in LS (low IS).

Table 1.Estimated top ten transcription factors (TFs) from the promoter region of DEGs expressed at different stages (FS vs. LS and LS vs. IS) in bovine endometrium

Comparing Stages	Estimated TFs	Descriptions	No. of DEGs with binding site
FS vs.LS			
Higher at FS	RFX	Regulatory Factor X	214
	RARA	Retinoic acid receptor alpha	162
	GRDBD	Glucocorticoid DNA binding domain	111
	RZR/ROR	Related orphan receptor	106
	MYF	Myogenic factor	83
Higher at LS	SP1	Specificity protein 1	210
	PRKCA	Protein kinase C alpha	108
	SP3	Specificity protein 3	77
	SP2	Specificity protein 2	76
	IRF1	Interferon regulatory factor 1	66
LS vs. IS			
Higher at IS	SP1	Specificity protein 1	86
	IRF9	Interferon regulatory factor 9	66
	IRF5	Interferon regulatory factor 5	43
	PRKCA	Protein kinase C alpha	36
	ZIC1	Zinc finger	28
Lower at IS	SP1	Specificity protein 1	175
	SP3	Specificity protein	95
	PRKCA	Protein kinase C alpha	91
	AP2	Activating protein 2	85
	HR	Lysine-specific demethylase hairless	79

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Identification of TFs from DEGs

Table 2 represents the comparison of estimated TFs and the TFs included in each DEG. In the group of FS vs. LS, 25 and 28 DEGs were expressed as TFs for highly expressed at FS and LS, respectively.

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DLX4 and IRF4 were included in both highly expressed DEGs and estimated TFs of specific at FS. CREG1, ELF5, GFI1, MYB and PPARG were included in both highly expressed DEGs at FS and estimated TFs of common with FS and LS.

While, there were no common TFs between DEGs of higher expression and specific TFs estimated from highly expressed DEGs at LS. EGR1, HNF1B, MTF1, POU5F1 and PPARA were included in both highly expressed DEGs at LS and estimated TFs of common with FS and LS.

On the other hand, 21 DEGs were expressed as TFs for highly expressed at IS. When compared it with estimated TFs of specific in highly expressed DEGs at IS, IRF5, IRF9, STAT1 and STAT2 were included in both groups.

Comparison of highly expressed TFs with estimated TFs of common with LS and IS, CREM, FOXS1 and IRF1 were included in both groups. There was no common TFs between DEGs of higher expression and estimated TFs of specific in lower expressed DEGs at IS.

In TFs included both in highly expressed and estimated TFs, DLX4 (40 DEGs), MYB (28), IRF5 (43), IRF9 (66) and IRF1 (32) had a large number of DEGs with binding site in the promoter region.

Table 2. Comparison of estimated TFs and TFs involved in DEGs at different stages (FS vs. LS and LS vs. IS) in bovine endometrium

Comparing Stages	No. of TFs in DEGs	No. of estimated TFs	No. of Common TFs	Common TFs	No. of DEGs with binding site
FS vs. LS					
Higher at FS	25	153	7	CREG1	6
				DLX4	40
				ELF5	4
				GFI1	2
				IRF4	11
				MYB	28
				PPARG	11
Higher at LS	28	156	5	EGR1	16
				HNF1B	15
				MTF1	1
				POU5F1	4
				PPARA	5
LS vs.IS					
Higher at IS	21	157	7	CREM	2
				FOXS1	7
				IRF1	31
				IRF5	43
				IRF9	66
				STAT1	15
				STAT2	2
Lower at IS	21	153	3*	CREM	2
				FOXS1	7
				IRF1	32

*Common TFs of highly expressed DEGs at IS and estimated TFs from the promoter region of DEGs in lower expression at IS.

Discussion

TFs in each stage were estimated from the promoter region of DEGs to understand the mechanism of gene expression in bovine endometrium. TFs contain a wide number of proteins which their functions are to initiate and control the transcription of genes for the production of functional proteins (Latchman. 1993). TFs in DEGs which expressed at the bovine endometrium were compared with estimated TFs.

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The results of current observation featured some unique TFs in bovine endometrium. CREG1, DLX4, ELF5, Gfi1, IRF4, Myb and PPARG recognized as a distinctive at FS. Cellular repressor of E1A-stimulated genes (CREG1) is a new and important glycoprotein that regulates tissue homeostasis.

However, small glycoprotein was initially described by Veal *et al* (1998) as a transcription repressor that antagonizes E1A-induced transcription activation. Our results showed that it is a small glycoprotein secreted outside the cell or reside at intracellular membrane compartments (Kunita*et al*. 2002).In this study, the CREG1 appeared in both DEG and estimated at FS and it would also be promoted by E2 and play an important role in gene expression in bovine endometrium at this stage.

Previous studies suggest that DLX4 is regulated by estrogen. In our study, DLX4 emerged in both DEG and estimated at FS and it would also be promoted via estrogen and play a key role in gene expression in bovine uterus at this stage.

E74-like factor 5 (ELF5) is an epithelial-specific member of the ETS transcription factor family (Oettgen*et al*.1999). A key role of ELF5 is the regulation of cell fate, beginning with specification of the trophectoderm in the blastocyst (Donnison*et al*. 2005).

ELF5 is in typical human tissues and reported to be expressed in the kidney, prostate, lung, mammary gland, salivary gland, placenta, and stomach (Oettgen*et al*.1999 and Zhou *et al*.1998).

Our results showELF5 emerged in both DEG and estimated at FS and it might be regulated by E2 and play an important role of gene expression in bovine endometrium at this stage.

Growth factor independence 1 (Gfi1) is a transcriptional repressor which is essential for the function and development of many different hematopoietic lineages. Gfi1 plays an essential role during granulocytic differentiation and characterized as a T cell oncogene (Zweidler *et al*.1996).

This gene is expressed in the common lymphoid progenitor and developing to T and B lymphocytes.However, during normal development, levels decrease when these cells are matured.

In mature hematopoietic cells, its expression is limited to granulocytes.Our finding shows that it is appeared in both DEG and estimated that it may play immune role against viruses and bacteria in bovine endometrium during FS.IRF4 belong to the IRF family of TFs has been regulated by estrogen (Carreras *et al*. 2010). It was also suggested that deregulation of IRF4 activities could exert more infected by pathogens in female (Carreras *et al*. 2010).

IRF4 is expressed precisely in lymphocytes and macrophage/dendritic cells (Honma *et al*. 2008). In thisresearch, IRF4 estimated as a specific TFs in FS which may have immune

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function against pathogens in bovine endometrium at FS.Mybis related to a large family of proteins, functionally diverse and represented in all eukaryotes.

Most Myb proteins have ability to work as TFs with varying numbers of Mybdomain and also they have ability to bind DNA directly (Ambawat*et al*. 2013). In mouse and human have been proposed to play essential roles in cell cycle control and in the proliferation and differentiation of hemopoietic cells.

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Myb is a sequence-specific DNA binding protein, which can either activate or repress transcription of different promoters (Graf. 1992). Thus, the function of Myb activate or repress TFs in different organisms.

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In our study,Myb emerged in both DEG and estimated at FS possibility has role at gene regulation during FS in bovine endometrium. Peroxisome proliferator-activated receptor gamma (PPARG) is mainly involved in the regulation of genes which are related to lipid metabolism and play a role in adipocyte differentiation and important TFs for adipogenesis (Ren *et al*.2002).

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PPARG belongs to the superfamily of nuclear receptors and those genes are activated by PPARG stimulate lipid uptake and adipogenesis by fat cells (Jones *et al*.2005).

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Therefore, the main role of PPARG is to control those genes which are responsible for lipid metabolism. In our finding, estimated PPARG TFs specific for FS in bovine endometrium. Furthermore, the PPARG should has role at gene regulation in bovine endometrium during FS.

In comparison of LS vs. FS, some TFs recognized as specific for LS in both new estimated and also in DEG andthey are EGR1, HNF1B, MTF1, POU5F1 and PPARA. Early growth response protein 1(EGR1) is a protein whichis encoded by *Egr1*and belongs to the EGR family of C2H2-type zinc-finger proteins. It is a nuclear protein and functions as a transcriptional regulator.

The target products of activated genes are required for differentiations and mitogenesis. EGR1 is rapidly prompted in several cell types by growth, differentiation and apoptotic stimuli such as growth factors, cytokines, hormones and environmental stresses (Lee*et al.*(1996) reported thatEGR1 is involved in follicular development, ovulation, luteinization and placental angiogenesis. EGR1deficient female mice were infertile due to lack of mature follicles, ovulation and luteinisation (Topilko*et al.* 1998). EGR1 TF regulated by progesterone hormone.

Similar in present study, EGR1 appeared in new estimated and DEG TF in bovine endometrium during LS and it will be playing an important role at gene expression in bovine endometrium during LS.The HNF1B gene offers guidelines for making a protein that binds to particular regions of DNA and controls activity of other genes.

On the basis of this function, the protein created from the HNF1B gene is named a TF. The HNF1B protein is part of a large group of TFs which is called homeodomain proteins. The homeodomain is an area of the protein that allows it to bind to DNA. The HNF1B protein is found in many organs and tissues including the lungs, liver, intestines, pancreas, kidneys, reproductive system, and urinary tract (Fague*et al.*2011).

HNF1B-associated are likely to be involved in regulating metabolic functions associated with renal cyst formation (Fendler*et al.*2016). HNF1B in human expressed in several organs such as kidney, uterus, prostate, stomach and etc. This is the first time that author estimated the HNF1B and its specific TFs in bovine endometrium. *Present study predicated the HNF1B is unique TFs I bovine endometrium during LS. Itmay have key role at gene expression during LS in bovine endometrium.*

Metal regulatory transcription factor 1 (MTF1) is a pluripotent transcriptional regulator involved in cellular adaptation to various stress conditions and primarily exposure to heavy metals but also to hypoxia or oxidative stress. MTF1 is evolutionarily preserved from mammals to insects and has been described for many species including human, mouse, capybara, pufferfish, zebrafish, trout and Drosophila melanogaster (Linder*et al.*2009).

MTF-1 is also involved in the transcriptional regulation of other metal-responsive genes such as zinc transporter 1 (Langmade*et al.* (2000recognized that the deficiency of MTF-1 in mouse embryo is lethal at day 14.They concluded MTF-1 also serves a developmental role.

Therefore, MTF-1 is a main TFs for regulate metals and it is playing a vital function in several species. Our results also show the MTF-1 in both new estimated and DEG in bovine identified during LS and it will be essential for gene expression in bovine endometrium at the period of LS.POU5F1 is a TF containing a POU homeodomain.

It plays a role in embryonic development (especially early embryogenesis) and is essential for embryonic stem cell pluripotency regulatory network (Simmet*et al.*2018). It shows thatit is a vital regulators of tissue-specific gene expression in lymphoid and pituitary differentiation

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and in early mammalian growth. To recognize members of the POU family of TFs,it may be involved in the tissue-specific regulation of genes expression in insulin-secreting cells of pancreas (Takeda *et al.*1992).

According to reference (Rajpert-De Meyts*et al.*2004), POU5F1 highly expressed in embryonic stem cells in some mammals. Our result indicates that POUF1 is specific TF during LS in bovine endometrium. Kim *et al* (2017) reported that POUF1 TF is controlled by reproductive hormone in human. During LS in bovine also level of progesterone is high and it may be controlled by progesterone and gene expression regulated in the endometrium.

Peroxisome proliferator-activated receptor alpha (PPARα) is a member of the steroid hormone receptor super family which is involved in the control of cellular lipid utilization. This makes PPARα as a candidate gene for type 2 diabetes and dyslipidemia (Vohl *et al.*2000). PPARs are members of a large family of ligand-inducible TFs that consist of receptors for retinoid, thyroid, and steroid hormones it controls the expression of target genes by binding to DNA sequence elements (Schoonjans*et al.*1996).

Analysis of PPAR mRNA distribution showed that the mammalian PPARa is predominantly expressed in tissues with high catabolic rates for fatty acids and peroxisomal metabolism such as liver, heart, kidney, intestinal mucosa, and brown adipose tissue, (Schoonjans*et al.*1996). The PPARs regulated by progesterone in human, monkey and rabbit. Our finding also shows PPARA appeared as specific TFs during LS in bovine endometrium and it may regulate through progesterone and play vital role at gene expression. Progesterone is a steroid hormone predominantly produced by the corpus luteum after ovulation and exerts its primary action by activating progesterone receptor (Adams *et al.*1997).

During LS and IS in bovine level of progesterone is high and it may have essential function at gene expression in bovine endometrium at LS and IS periods.The transcriptional activity of interferon regulatory transcription factor-1 (IRF1) is dramatically upregulated by viral infection and stimulated by proinflammatory cytokines, chemokines and prostaglandins which mediated by infection and inflammation (Yarilina*et al.*2008).

IRF1 is a positive regulatory transcription factor that binds to a common motif in the promoter region of interferons and several IFN-inducible genes which include double-stranded RNA-dependent protein kinase (Penninger *et al.*1997). IRF1 is necessary for the development of natural killer cells and the differentiation of CD8+T cells.

IRF1 controls gene expression in developing thymocytes and it is required for lineage commitment and selection of CD8+thymocytes. Mice lacking IRF1 displayed reduced numbers of mature CD8+T cells within the thymus and peripheral lymphatic organs (Penninger *et al.*1997). IRF1 is responsible for the important factors expression of innate responses and the development and function of adaptive immunity.

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The IRF groups are cytokine and inhibit the activity of virus and bacteria and they are mostly initiatedduring pregnancy. In contrast,our study recognized that IRF1 appeared as TF and bind with the large number of DEG. Perhaps the IRF1 has role at gene expression in bovine endometrium during LS and there is possibility that IFN type I-II present during LS.

Hepatocyte nuclear factor 3 (HNF3) is a protein and it may have a limited tissue distribution than the other two factors reported previously and showing the limited distribution appearance based on transcriptional control of the HNF-3A gene. The HNF3 DNA binding activities were purified from rat liver extracts and the rat cDNA subsequently cloned and sequenced.

The rat HNF3 genes have been expressed not only in liver but also in stomach, intestine and lung which are all tissue derived at least partly from embryonic endometrium. HNF3 gene is important in early endoderm and liver development and in addition to their role in adult liver transcription (Kaestner*et al.*1994). The HNF3 mostly expressed in liver and also in rat reported expressed other organs.

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For first time, present study estimated that HNF3 is a TF in bovine endometrium during LS. According to reference Azmi *et al*(2013), HNF3 and progesterone have positive correlation in human. There is possibility in bovine endometrium that HNF3 regulatesthrough progesterone during LS with high level of progesterone in bovine.In another hand, serum response factor (SRF) is a member of superfamily of TFs.

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Furthermore, comparing IS DEG TFs with IS estimated TFs displayed STAT1, STAT2, IRF5, and IRF9 in both groups. Moreover, STAT1 and STAT2 remained persistently phosphorylated and also they were in the nucleus upon long-term stimulation of cells with IFNt (Stewart *et al.*2001).

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STAT1 and STAT2 proteins are essential intermediator of type I and III IFN signalling, those which are reported as a necessary factor of the cellular antiviral functions and they are also the primary element of the TFs compound in the IFN signaling pathways(Au-Yeung*et al.* 2013) IRF5 is critical TFs in the type I IFN pathway and control the expression of IFN dependent genes (Yanai *et al.* 2007).

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It encourages gene transcription by binding to target DNA sequence such as ISRE in the cis-regulatory area of target genes (Barnes *et al.* 2002). IRF9 is playing intermediator role among STAT1,STAT2 and ISRE (Yanai *et al.* 2012). The tyrosine phosphorylation of STAT1 produced by IFNT leads to the formation of STAT1, STAT2 and IRF9 heterodimers and it also identified as IFN-stimulated gene factor 3 complex (ISGF3), which is translocated to the nucleus and attach to the interferon-stimulated response element which exist within the promoter of ISGs and controls their expression (Basavaraja*et al.* 2017). In present study, STAT1, STAT2, IRF5 and IRF9 estimated as specific TFs at IS and according to previous reports, they have key function to gene expression in bovine endometrium during IS.

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