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Early transcriptome responses of the bovine midcycle corpus luteum to prostaglandin F2α includes cytokine signaling

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ABSTRACT

In ruminants, prostaglandin F2alpha (PGF2α)-mediated luteolysis is essential prior to estrous cycle resumption, and is a target for improving fertility. To deduce early PGF2α-provoked changes in the corpus luteum a short time-course (0.5–4 h) was performed on cows at midcycle. A microarray-determined transcriptome was established and examined by bioinformatic pathway analysis. Classic PGF2α effects were evident by changes in early response genes (FOS, JUN, ATF3) and prediction of active pathways (PKC, MAPK). Several cytokine transcripts were elevated and NF-κB and STAT activation were predicted by pathway analysis. Self-organizing map analysis grouped differentially expressed transcripts into ten mRNA expression patterns indicative of temporal signaling cascades. Comparison with two analogous datasets revealed a conserved group of 124 transcripts similarly altered by PGF2α treatment, which both, directly and indirectly, indicated cytokine activation. Elevated levels of cytokine transcripts after PGF2α and predicted activation of cytokine pathways implicate inflammatory reactions early in PGF2α-mediated luteolysis.

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1. Introduction

In mammals, multiple fertile cycles depend on the formation and regression of a transient endocrine structure in the ovary termed the corpus luteum (CL) (Meidan, 2017). The CL forms during each estrous cycle and synthesizes progesterone, a hormone critical for early embryonic survival during pregnancy (Micks et al., 2015; Spencer et al., 2016). However, before the next follicle can develop, the steroidogenic luteal cells of the CL must cease progesterone production—contingent on the absence of a pregnancy—and ultimately undergo apoptosis (Aboelenain et al., 2015; Del Canto et al., 2007). Prostaglandin F2alpha (PGF2α) is a recognized lipid mediator that triggers luteal regression after an unsuccessful reproductive cycle or at parturition in mammals (Davis and Rueda, 2002). Thus, PGF2α-mediated luteolysis is a key checkpoint in the reproductive cycle and is a useful target for controlling the estrous cycle and fertility.

Signaling by PGF2α has been studied extensively *in vitro*, and the classic signaling pathway involves the binding of PGF2α to its G-protein coupled receptor and activating $G\alpha_{q/11}$ (McCann and Flint,

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Abbreviations

Protein/*Gene*
ABCA1 ATP binding cassette subfamily A member 1
ABCG1 ATP binding cassette subfamily G member 1
APOA1 apolipoprotein A1
APOE apolipoprotein E
ATF3/*ATF3* activating transcription factor 3
CCL/*CCL* C-C motif chemokine
CH25H/*CH25H* cholesterol 25-hydroxylase
CL corpus luteum
CYP11A1 cytochrome P450 family 11 subfamily A member 1
EDN1 endothelin 1
EGR/*EGR* early growth response protein 1/3/4
ERK extracellular signal-regulated kinase
FOS/*FOS* Finkel-Biskis-Jinkins murine osteosarcoma viral oncogene homolog
GEO Gene Expression Omnibus
HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HSD3B1 hydroxyl-δ-5-steroid dehydrogenase, 3 β and steroid δ-isomerase 1
HSL/*LIPE* Hormone sensitive lipase, type E
IL-/*IL* interleukin
INSIG1/*INSIG1* insulin induced gene 1
IPA Ingenuity Pathway Analysis
JUN/*JUN* Jun proto-oncogene
LDLR low density lipoprotein receptor
LDLRAP1/*LDLRAP1* low density lipoprotein receptor adaptor protein 1
LHCGR Luteinizing hormone/chorionic gonadotropin receptor
LLC large luteal cells
MAPK mitogen-activated protein kinase
mRNA messenger RNA
NF-κB/*NFKB1* nuclear factor kappa B
NR1H nuclear receptor family 1 subfamily H member 2/3
NR4A/*NR4A* nuclear receptor subfamily 4 group A member 1/2/3
PGF2α prostaglandin F2alpha
PKC/*PKCD* protein kinase C
qPCR quantitative real-time PCR
RMA robust multi-array average
SCARB1 scavenger receptor class B member 1
SEM standard error of the mean
SLC small luteal cells
SOM self-organizing map
StAR/*StAR* steroidogenic acute regulatory protein
STAT signal transducer and activator of transcription 1/3
TGFβ/*TGFB* transforming growth factor beta 1/2
TNFα/*TNF* tumor necrosis factor alpha
USF1 upstream transcription factor 1
VEGF vascular endothelial growth factor A

1993; Väänänen et al., 1998). The early intracellular signaling events initiated by PGF2α in luteal cells include the activation of phospholipase C (Davis et al., 1987), phospholipase A2 (Kurusu et al., 2012, 1998), an increase in intracellular Ca^{2+} (Davis et al., 1987), activation of protein kinase C (PKC) (Chen et al., 2001) and activation of mitogen-activated protein kinase (MAPK) signaling cascades including extracellular signal-regulated kinase (ERK) (Arvisais et al., 2010; Chen et al., 2001, 1998; Yadav and Medhamurthy, 2006). These signaling cascades are responsible for the induction of several early response genes including, Finkel-Biskis-Jinkins murine osteosarcoma viral oncogene homolog (*FOS*) (Chen et al., 2001), Jun proto-oncogene (*JUN*) (Chen et al., 2001), early growth response 1 (*EGR1*) (Hou et al., 2008), and activating transcription factor 3 (*ATF3*) (Mao et al., 2013). These initial alterations will trigger changes in the CL proteome enabling luteolysis to proceed. For example, EGR1 expression stimulates the synthesis of transforming growth factor beta (TGFβ) (Hou et al., 2008), which can inhibit luteal progesterone secretion (Hou et al., 2008), act on luteal endothelial cells to disrupt the microvasculature (Maroni and Davis, 2011), and stimulate the profibrotic activity of luteal fibroblasts (Maroni and Davis, 2012).

The luteolytic process is a well-coordinated series of events similar to an acute inflammatory response consisting of a sequential time-dependent infiltration of neutrophils, macrophages, and T lymphocytes (Best et al., 1996; Penny et al., 1999). Accordingly, there is likely time-dependent secretion of cytokines to recruit and activate the various leukocytes (Townson and Liptak, 2003). Several cytokine transcripts are induced by PGF2α in the mid-to late-stage CL including tumor necrosis factor alpha (*TNF*) (Shah et al., 2014), interleukin 1 beta (*IL1B*) (Atli et al., 2012; Mondal et al., 2011), *TGFB1* (Hou et al., 2008; Mondal et al., 2011; Shah et al., 2014), and the chemokines; C-C motif chemokine ligand 2 (*CCL2*, previously known as *MCP1*) (Mondal et al., 2011; Penny et al., 1998) and C-X-C motif 8 (*CXCL8*, previously known as *IL8*) (Atli et al., 2012; Mondal et al., 2011; Shah et al., 2014; Shirasuna et al., 2012b; Talbott et al., 2014). These cytokines have pleiotropic effects on luteal cells, including inhibition of progesterone secretion, stimulation of PGF2α secretion, and stimulation of apoptosis of multiple luteal cell types (Pate et al., 2010). The production of luteolytic factors, decrease in progesterone secretion, recruitment of immune cells, release of pro-inflammatory cytokines, reduction in blood supply (Shirasuna, 2010), and the creation of a hypoxic environment (Nishimura and Okuda, 2015) likely act in concert within the CL to cause the functional and structural regression of the CL.

The purpose of this study was to understand the early PGF2α-elicited changes in the CL based on temporal patterns of early transcript expression following *in vivo* treatment with PGF2α. While many studies have examined luteolytic alterations both *in vivo* and *in vitro*, most studies have focused on changes 3–24 h after PGF2α administration (Mondal et al., 2011; Shah et al., 2014) or used targeted rather than global approaches (Atli et al., 2012; Shirasuna et al., 2012a, 2010). Therefore, little is known about the very early temporal changes in global mRNA expression elicited in response to PGF2α treatment *in vivo*. In the present study, a systems biology approach using Affymetrix Bovine microarray was employed to evaluate gene expression at 0.5–4 h after PGF2α; followed by bioinformatics analysis of PGF2α-mediated signals. We hypothesized that the sequence of events after *in vivo* PGF2α administration would include early changes of classical targets of PGF2α signaling pathways followed by fluctuations in targets of cytokine signaling at later times.

2. Materials and methods

2.1. Animals

Postpubertal multiparous female cattle (n = 15) of composite breeding (½ Red Angus, Pinzgauer, Red Poll, Hereford and ½ Red