Thrombospondin-1 at the crossroads of corpus luteum fate decisions

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Abstract

The multimodular matricellular protein thrombospondin-1 (THBS1) was among the first identified endogenous antiangiogenic molecules. Recent studies have shown THBS1-mediated suppression of angiogenesis and other critical activities for corpus luteum (CL) regression. THBS1 is specifically induced by prostaglandin F2alpha in mature CL undergoing regression, whereas luteinizing signals such as luteinizing hormone and insulin reduced its expression. THBS1 interacts both synergistically and antagonistically with other essential luteal factors, such as fibroblast growth factor 2, transforming growth factor beta1 and serpin family E member 1, to promote vascular instability, apoptosis and matrix remodeling during luteal regression. Expression of THBS1 is also downregulated by pregnancy recognition signals to maintain the CL during early pregnancy. This dynamic pattern of luteal expression, the extensive interactivity with other luteal factors and strong antiangiogenic and proapoptotic activities indicate that THBS1 is a major determinant of CL fate.

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Introduction

The corpus luteum (CL) is a transient endocrine gland that develops from the preovulatory follicle after ovulation. A functional CL is critical for successful mammalian reproduction. Extension of luteal lifespan and secretion of progesterone are required for establishing and maintaining pregnancy. Conversely, regression of the CL in the absence of a viable embryo is necessary for the initiation of a new reproductive cycle. The CL lifespan is characterized by rapid growth, differentiation and controlled regression, phases accompanied by intense angiogenesis, stabilization and angioregression, respectively (Niswender *et al.* 2000, Schams & Berisha 2004, Shirasuna *et al.* 2012). Prostaglandin F2alpha (PGF2a) is the principal luteolytic hormone in cattle and many other species with an estrous cycle. It is secreted by the uterus in a non-fertile cycle (McCracken *et al.* 1999, Niswender *et al.* 2000). Endogenous PGF2a or its exogenous administration initiates a cascade of events leading to the irreversible demise of the CL. During this process, the CL undergoes dramatic changes in its steroidogenic capacity, vascularization, extracellular matrix (ECM) remodeling and cell viability (Schams & Berisha 2004, Miyamoto *et al.* 2010, Meidan *et al.* 2017). An intriguing question concerning bovine luteal function is its refractoriness to the luteolytic actions of PGF2a in the early luteal phase, which lasts up to day 5 of the bovine estrous cycle (Lauderdale 2009).

To obtain a better understanding of early CL resistance to PGF2a-induced luteolysis, Mondal *et al.* compared the transcriptome of PGF2a-responsive bovine CL on day 11 of the estrous cycle to that of PGF2a-refractory CL on day 4, both before and at two time points (4 and 24 h) after PGF2a administration (Mondal *et al.* 2011). This study revealed a potential functional relationship between angiogenesis and the luteolytic response to PGF2a. PGF2a markedly increased expression of the proangiogenic factor fibroblast growth factor 2 (FGF2) in the PGF2a-refractory (day 4) CL. In contrast, antiangiogenic genes were specifically and highly induced in the PGF2a-responsive (day 11) CL. These antiangiogenic genes included thrombospondin 1 and 2 (THBS1 and 2), transforming growth factor beta 1 (TGFB1) and serpin family E member 1 (SERPINE1) (Mondal *et al.* 2011, Berisha *et al.* 2016, Farberov & Meidan 2016). The present review summarizes current knowledge on the regulation of THBS1 expression and its role in cell survival and blood vessel dynamics within the CL.

The thrombospondin family

The thrombospondins are a family of five conserved extracellular multidomain glycoproteins. Members of this family play essential roles in regulating cell–cell and cell–matrix interactions (Bornstein 2001). These proteins can be subdivided into two structural groups: subgroup

A comprising THBS1 and THBS2, which have the same set of structural domains and subgroup B comprising THBS3–THBS5, which are shorter and lack both regions related to procollagen type I repeats and the fourth type II repeat (Adams & Lawler 2011).

THBS1 and THBS2 were among the first endogenous protein inhibitors of angiogenesis to be identified (Bornstein 2009). Knockout mice show increased vascularization (Lawler *et al.* 2001, Wang *et al.* 2003), suggesting that THBS1 inhibits angiogenesis. Although THBS1 and THBS2 are structurally similar, they differ in their expression patterns, localization and some functions (Lawler 2000, Bornstein 2001, Adams & Lawler 2004). Indeed, THBS1- and THBS2-knockout mice have distinct phenotypes (Adams & Lawler 1993, Kyriakides *et al.* 1998, Lawler *et al.* 1998).

THBS1 is a 420kDa homotrimer; each monomer consists of an N-terminal globular module followed by a coiled-coil oligomerization domain, a procollagen module, three properdin-like type I repeats (TSR1), epidermal growth factor (EGF)-like type II repeats (TSR2), type III repeats (TSR3) and a C-terminal globular domain (Carlson *et al.* 2008).

Each domain of THBS1 interacts specifically with different molecules. The N-terminal domain interacts with low-density lipoprotein receptor-related protein (LRP1), heparin sulfate proteoglycans (HSPGs) and a number of integrins (Tan *et al.* 2006). Adjacent to the N-terminal, there is a region that is homologous to procollagen. This domain is involved in the assembly of the protein into a trimer (Sottile *et al.* 1991). All five members of the THBS family have the repeat domain types II and III, but only THBS1 and THBS2 contain the type I repeats. The type I repeat region is the most studied antiangiogenic epitope of THBS1 as it mediates interactions with the CD36 receptor, fibronectin, HSPGs and latent TGF β 1 (Jimenez *et al.* 2000, Murphy-Ullrich & Poczatek 2000, Resovi *et al.* 2014). The type II repeats mediate cell adhesion by binding β 1 integrins (Calzada *et al.* 2004). The type III repeats contain the integrin recognition motif RGD (Kvansakul *et al.* 2004) and a binding site for FGF2 (Colombo *et al.* 2010). This binding site inhibits angiogenesis by sequestration of FGF2 (Colombo *et al.* 2010, Pagano *et al.* 2012). The COOH-terminal domain of THBS1 binds CD47, also known as an integrin-associated protein (Brown & Frazier 2001). Therefore, owing to its structural and functional domains, THBS1 can simultaneously bind to cell-surface receptors, ECM components, proteolytic enzymes, cytokines and growth factors.

Hormonal regulation and expression patterns of thrombospondins in the ovary

THBS1, THBS2, and their receptors (CD36 and CD47) are widely expressed in the ovary, including granulosa

cells (GCs), luteal steroidogenic cells and endothelial cells (ECs) (Dreyfus *et al.* 1992, Higuchi *et al.* 1999, Petrik *et al.* 2002, Zalman *et al.* 2012, Farberov & Meidan 2014, Berisha *et al.* 2016). Studies in rat, bovine, marmoset and human have confirmed that within the follicle, THBSs are primarily produced in the GC layer (Dreyfus *et al.* 1992, Higuchi *et al.* 1999, Petrik *et al.* 2002, Greenaway *et al.* 2005, Thomas *et al.* 2008). Upon differentiation by FSH and forskolin, the ability of GCs to produce and bind THBS1 is inhibited (Dreyfus *et al.* 1992). In agreement, a recent study reported that *in vitro* luteinization by LH plus insulin reduced expression levels of THBS1, THBS2 and CD36 in bovine GCs (Fig. 1) (Farberov & Meidan 2014). However, studies in rodents reported increased expression of *Thbs1* and *Thbs2* in developing CL immediately after ovulation (Bagavandoss *et al.* 1998, Petrik *et al.* 2002). A possible explanation for this seeming paradox of high THBS expression in a period of rapid vascular growth is that this increase is not hormone induced but rather due

Figure 1 Hormonal regulation of thrombospondin (THBS) expression in ovarian cells. Follicular maturation induced by follicle-stimulating hormone (FSH) and luteinization stimulated by luteinizing hormone (LH) and insulin decrease expression of THBS1 and THBS2 in bovine granulosa cells (GCs). Treatment with prostaglandin F $_{2\alpha}$ (PGF $_{2\alpha}$ treatment) on the other hand elevates the expression of THBS1 and THBS2 in both luteal ECs and GCs.

to a massive migration of ECs into the central region of the developing CL. This contention is supported by THBS1 immunostaining in the forming CL (days 1–2) showing weak THBS1 expression in large luteal cells and strong expression in capillary ECs (Berisha *et al.* 2016). THBS1, THBS2, CD36 and CD47 are colocalized in follicles, suggesting that THBS ligands and receptors may contribute to the autocrine control of follicular cell function (Higuchi *et al.* 1999, Petrik *et al.* 2002, Greenaway *et al.* 2005, Berisha *et al.* 2016).

In the CL, THBSs and their receptors exhibit cell-specific expression. *THBS1* is the predominant form expressed in luteal ECs during early and midluteal stages (Zalman *et al.* 2012, Berisha *et al.* 2016), whereas *THBS2* is the major form in luteinized granulosa cells (LGCs) (Farberov & Meidan 2014). *CD36* expression was found only in LGCs, whereas *CD47* expression did not display an appreciable difference in the expression level between these two cell types (Farberov & Meidan 2014).

THBSs and their receptors are dynamically expressed across the luteal lifespan in cow and sheep ovary (Zalman *et al.* 2012, Romero *et al.* 2013, Berisha *et al.* 2016). Berisha *et al.* (2016) found that mRNA expression levels of *THBS1*, *THBS2* and *CD47* in the CL were high and relatively stable during the estrous cycle, whereas *CD36* expression precipitously increased in the regressing CL. In general, the ability of the bovine CL to undergo PGF2a-induced luteolysis is correlated with the upregulation of *THBS1*, *THBS2* and *CD36* (Zalman *et al.* 2012, Berisha *et al.* 2016, Farberov & Meidan 2016). Notably, PGF2a treatment *in vitro* upregulated *THBS1* and *THBS2* expression in both luteal ECs and LGCs (Fig. 1) (Zalman *et al.* 2012). Likewise, *CD47* displayed a significant and persistent increase in the mature (day 11) CL at 4 and 24 h after PGF2a administration (2.2-fold relative to the 0 time point) (Farberov & Meidan, unpublished data). The expression of *CD47* was also induced in cultured LGCs by PGF2a treatment (Farberov & Meidan 2014). In contrast to cyclic CL, expression levels of THBSs and receptors were downregulated for the whole duration of pregnancy (Berisha *et al.* 2016). Furthermore, studies in ewes showed an increase in *THBS1* mRNA in regressing CL that was inhibited by early pregnancy (Romero *et al.* 2013). Interferon tau (IFNt), a maternal pregnancy recognition factor secreted during early pregnancy by the conceptus, exerts endocrine actions in the CL (Bott *et al.* 2010, Romero *et al.* 2015, Basavaraja *et al.* 2017). It was recently reported that the expression of *THBS1* was downregulated in bovine CL slices and cultured luteal ECs incubated with IFNt (Basavaraja *et al.* 2017). The same study also found that IFNt reduced *TGFB1* and *SERPINE1*, which are interconnected with THBS1, during luteolysis (Farberov & Meidan 2016, Meidan *et al.* 2017). Inhibition of the THBS1–TGFB1–SERPINE1 feed-forward process may be critical for maintaining the CL during early pregnancy.

The temporal expression and localization patterns of THBSs and their receptors during follicular maturation and across luteal lifespan are compatible with inhibitory functions in ovarian angiogenesis.

THBS1 promotes apoptosis of ovarian cells

The induction of apoptosis by THBS1 is essential for its antiangiogenic activity (Jimenez *et al.* 2000). THBS1 was found to inhibit angiogenesis in whole rat follicles *in vitro* and to directly induce apoptosis of GCs *in vitro* (Garside *et al.* 2010a). A study in marmoset monkeys showed that *in vivo* treatment with ABT-898 (a mimetic peptide of THBS1 type I repeats (Garside *et al.* 2010b)) reduced endothelial cell proliferation in the thecal layer of preantral and early antral follicles and suppressed GCs proliferation in developing follicles. This treatment also increased the incidence of atresia in preantral and early antral follicles (Garside *et al.* 2010b). The expression levels of THBSs and associated receptors decrease as follicular development progresses (Petrik *et al.* 2002, Greenaway *et al.* 2005, Thomas *et al.* 2008, Berisha *et al.* 2016), while THBS1 is upregulated during atresia (Thomas *et al.* 2008).

Recent studies in bovine luteal cells have utilized RNA interference to show that THBS1 compromises the viability of both luteal ECs and LGCs (Farberov & Meidan 2014). Studies using a siRNA to downregulate endogenous THBS1 expression have providing evidence for a local proapoptotic effect of THBS1 in luteal cells. Cleaved caspase-3 levels were significantly reduced in THBS1-silenced luteal ECs as well as LGCs. Furthermore, silencing of THBS1 in luteal ECs rendered these cells more competent to withstand cell death induced by exposure to staurosporine (Farberov & Meidan 2014). These findings suggest that endogenous THBS1 acts as a proapoptotic compound in luteal cells. It is widely accepted that apoptosis occurs during structural luteolysis (Juengel *et al.* 1993, Rueda *et al.* 1999, Yadav *et al.* 2005), and these findings strongly suggest that THBS1 is essential for the apoptotic demise of the CL.

Several studies have reported that the antiangiogenic effects of THBS1 are mediated, at least partially, by the CD36 receptor. Indeed, CD36 activation triggers a signaling cascade leading to endothelial cell death by apoptosis and the collapse of tumor vessels (Jimenez *et al.* 2000, Nicholson *et al.* 2001, Simantov *et al.* 2005). However, among the various bovine luteal cells, this receptor was found to be expressed mainly in LGCs (Farberov & Meidan 2014). Surprisingly, recombinant THBS1 and the CD36-binding peptide ABT898 (Garside *et al.* 2010b) had only modest effects on bovine LGCs viability. In contrast, despite the lack of CD36 receptors in luteal ECs, 500 ng/mL THBS1 decreased LGC numbers by 80% and promoted the

activation of caspase-3. As expected, ABT-898 treatment did not affect the viability of luteal ECs (Farberov & Meidan 2014). These findings suggest that THBS1 acts in luteal ECs through a CD36-independent mechanism.

Unlike CD36, the CD47 receptor is expressed in both luteal ECs and LGCs (Farberov & Meidan 2014). The interaction of THBS1 with CD47 induced apoptosis in human umbilical vein ECs (HUVECs) and microvascular ECs (Freyberg *et al.* 2000, Xing *et al.* 2009). However, silencing of CD47 by siRNA did not rescue luteal ECs from THBS1-induced cell death (Farberov & Meidan, unpublished data), suggesting that CD47 may not contribute to the inhibitory effects of THBS1 in luteal ECs. Another possible mechanism underlying the apoptotic actions of THBS1 in luteal cells is its ability to interact with various integrins (Calzada *et al.* 2004). It was found that THBS1 is a ligand for alpha 9 beta1 integrin and that the integrin binding site is located within the N-terminal domain of THBS1. The N-terminal domain of THBS1 is also implicated in the proangiogenic activity of this molecule in microvascular ECs (Staniszewska *et al.* 2007). Another integrin, alpha 3 beta 1 integrin, was identified as an additional receptor for THBS1. This interaction could either inhibit or stimulate endothelial cell proliferation depending on cell origin (Chandrasekaran *et al.* 2000).

Another alternative pathway by which THBS1 may affect luteal endothelial cell viability is through its HSPG-binding domain (Chen *et al.* 2000). Interaction of THBS1 with HSPGs inhibits mitogenesis and proliferation of aortic and corneal ECs (Vogel *et al.* 1993). Binding of THBS1 to HSPGs results in the transmission of intracellular signals that induce apoptosis in myeloid leukemia cells (Bruel *et al.* 2005). HSPGs are widely expressed in ovarian GCs, and their synthesis, internalization and release are regulated by gonadotrophins (Yanagishita 1994). Immunolabeling for HSPG is very intense in luteal cells and capillary ECs of mature CL (Hasan *et al.* 2002), supporting a role in THBS1 action; however, this notion has not been examined experimentally in ovarian cells.

Interaction of THBS1 with proangiogenic FGF2

THBS1 is known to inhibit angiogenesis both directly and indirectly by interacting with growth factors, cytokines and proteases. One such interaction is with FGF2, a vital proangiogenic factor in the CL (Robinson *et al.* 2007, Yamashita *et al.* 2008). By binding to the FGF2 heparin-binding domain, THBS1 prevents the interaction of FGF2 with HSPGs on the cell surface and in the ECM (Fig. 2) (Colombo *et al.* 2010), which ultimately reduces FGF2 bioavailability and activity (Pagano *et al.* 2012) (Fig. 2). FGF2 is not only proangiogenic but also sustains steroidogenic cell survival and stimulates progesterone secretion when infused into bovine CL (Liebermann *et al.*

Figure 2 Schematic representation of THBS1–FGF2 interaction. Binding of FGF2 to FGFR with heparin sulfate proteoglycan (HSPG) as a cofactor induces the formation of the ternary FGF2–FGFR–HSPG complex. This complex activates the receptor tyrosine kinase domain by phosphorylating tyrosine residues. The activated receptor triggers multiple downstream signaling pathways that drive cell proliferation, migration, and survival. Type III repeats within the THBS1 sequence sequester FGF2, preventing its interaction with HSPG in the extracellular matrix and on the cell surface. Thus, THBS1 affects FGF2 internal dynamics, causing allosteric inhibition of FGF2 binding to its cognate tyrosine kinase receptors and leading to inhibition of FGF2 angiogenic activity.

1996). Studies in bovine luteal ECs showed that THBS1 and small-molecule 27, which mimics the FGF2-binding site of THBS1, inhibit FGF2-induced proliferation and migration *in vitro* (Zalman *et al.* 2012). Consistent with these results, THBS1 inhibited the phosphorylation of p44/p42 mitogen-activated protein kinase (MAPK; ERK1/2) induced by FGF2 in luteal ECs (Farberov & Meidan, unpublished data). Furthermore, siRNA ablation of THBS1 elevates the levels of phosphorylated p44/42 (unpublished data). The antagonistic actions of FGF2 and THBS1 were further demonstrated by the ability of FGF2 to reduce basal and THBS1-induced caspase-3 levels in ECs derived from the CL (Farberov & Meidan 2014).

Notably, *THBS1* and *FGF2* expression in bovine CL exhibited the most divergent induction profile by PGF2a. Whereas *FGF2* was robustly induced in young CL (day 4) and much less in responsive CL (day 11), *THBS1* was prominent in the PGF2a-responsive CL (day 11) (Zalman *et al.* 2012). These expression profiles suggest reciprocal regulation of FGF2 and THBS1 in luteal cells by luteinizing signals and PGF2a. This proposition was confirmed by findings demonstrating that *THBS1* expression in cultured LGCs was inhibited by luteinizing

signals (LH and insulin), whereas *FGF2* expression was elevated after LH treatment and by PGF2a (Farberov & Meidan 2014) (Fig. 1). Moreover, these two proteins are mutually inhibitory at the gene expression level, that is, THBS1 added exogenously to luteal ECs dose dependently suppresses *FGF2* expression and vice-versa (Fig. 3) (Farberov & Meidan 2014). In agreement, THBS1 silencing elevated *FGF2* levels in both luteal ECs and LGCs (Farberov & Meidan 2014). Suppression of *THBS1* expression by FGF2 was also reported in mouse breast cancer cells, but the regulatory mechanisms were not examined (Mattila *et al.* 2006). Intracellular responses to FGF2 are mediated by several signal transduction cascades, including the RAS-MAPK, PI3K and phospholipase C pathways (Dailey *et al.* 2005). The RAS-MAPK pathway regulates the expression of diverse target genes through activation of E26 transformation-specific (ETS) transcription factors (Tsang & Dawid 2004), and several ETS sites are present in the *THBS1* promoter, suggesting FGF2-induced *THBS1* repression through RAS-MAPK signaling (Zabuawala *et al.* 2010). Activation of the PI3K/AKT pathway may also contribute to FGF2-mediated inhibition of *THBS1* synthesis (Bussolati *et al.* 2006, Kim *et al.* 2006). Furthermore, FGF2 can suppress *THBS1* expression via upregulation of two repressors, YY-1 (Yin Yang-1) (Santiago *et al.* 2001) and Id-1 (inhibitor of differentiation-1) (Passiatore *et al.* 2011), which were reported to bind to the *THBS1* promoter (Volpert *et al.* 2002, Kang *et al.* 2004).

The results described here suggest a transcriptional and functional interplay between THBS1 and FGF2 (Figs 2 and 3). This relationship is expected to regulate angiogenesis in a luteal stage-specific manner.

Figure 3 Proposed THBS1 network of interactions with FGF2, TGFB1 and SERPINE1 in luteal cells. THBS1 is a potent antiangiogenic factor that can directly disrupt the angiogenic properties of luteal ECs through multiple mechanisms. THBS1 also inhibit the proangiogenic actions of FGF2. These two proteins are mutually inhibitory at the gene expression level. In addition, THBS1 activates latent TGFB1, thus enabling TGFB1 signaling, which results in upregulation of *SERPINE1* (encoding PAI-1). PAI-1 further promotes vascular instability. Active TGFB1 can also enhance its own mRNA expression and that of THBS1, a process that would lead to further induction of *SERPINE1*.

THBS1–TGFB1 axis

TGFB family members are produced as inactive complexes with latency-associated peptide (LAP) and must be activated by molecules that facilitate release from the latent complex (Robertson & Rifkin 2013). THBS1 is the only member of the THBS family that can activate TGFB1 (Figs 3 and 4) (Murphy-Ullrich & Poczatek 2000).

A unique motif, KRFLK, situated in the type I repeats domain of THBS1 can physically interact with the LSKL sequence in LAP, resulting in the release of active TGFB1 (Ribeiro *et al.* 1999). The corresponding peptide from THBS2 (TRIR) does not activate TGFB1 (Murphy-Ullrich & Poczatek 2000) (Fig. 4). Once activated, TGFB1 can bind to its cell membrane receptors (TGFBRI and TGFBRII), leading to phosphorylation of SMAD2 and SMAD3 proteins, which in turn complex with SMAD4, translocate to the nucleus and bind to SMAD response elements that regulate target gene expression (Fig. 4) (Massague 2000). Like THBS1, *TGFB1* expression was induced only in mature (day 11) CL, after PGF2a injection (Mondal *et al.* 2011, Farberov & Meidan 2016). Expression of the TGFB1 downstream target *SERPINE1* (Boehm *et al.* 1999) was also markedly upregulated by PGF2a only in day 11 CL undergoing luteolysis (Fig. 4) (Farberov & Meidan 2016). Upregulation of *SERPINE1* by luteolytic signals was also shown in ovine CL (Romero *et al.* 2013). Recently, it was demonstrated that THBS1 can activate latent TGFB1 in bovine luteal ECs (Farberov & Meidan 2016). THBS1 elevated *SERPINE1* expression in these cells, an effect that was abolished by a TGFBRI kinase inhibitor. Additionally, THBS1 markedly increased the effects of latent TGFB1 on SMAD2 phosphorylation (phospho-SMAD2) and *SERPINE1* upregulation. THBS1 silencing also resulted in reduced *SERPINE1* mRNA and phospho-SMAD2 levels. Lastly, THBS1 actions on *SERPINE1* were inhibited partially by LSKL peptide (Farberov & Meidan 2016) (Fig. 4).

The role of TGFB1 in apoptotic cell death is context dependent (Sanchez-Capelo 2005). In bovine luteal ECs, TGFB1 promotes apoptosis (Maroni & Davis 2011, Farberov & Meidan 2016), limits cell growth and disrupts capillary morphogenesis and endothelial barrier function (Maroni & Davis 2011). TGFB1 also reduces the viability of cells and activates caspase-3, whereas inhibition of TGFBRI eliminates this proapoptotic effect (Farberov & Meidan 2016). Importantly, although THBS1 can activate TGFB1, the proapoptotic effect of THBS1 is not mediated via TGFB1 in luteal ECs. THBS1 is a significantly more potent inhibitor of cell viability than TGFB1, and this effect is not abolished by a TGFBRI inhibitor (Farberov & Meidan 2016).

In addition to the SMAD-mediated canonical TGFB1 signaling pathway, TGFB1 may signal through all three MAPK pathways, extracellular signal-regulated kinases, c-Jun N-terminal kinases and p38 MAPK (Zhang 2009).

Figure 4 Activation of TGFβ1 signaling pathway by THBS1. TGFβ1 is produced in a latent form consisting of the mature TGFβ1 dimer and a non-covalently related latency-associated peptide (LAP). Activation by THBS1 involves a WXXW motif in type I repeats for binding to the mature TGFβ1 dimer and a proposed second recognition sequence, KRFK, which binds to a conserved sequence (LSKL) near the amino-terminus of LAP. These sequences disrupt the non-covalent interactions between LAP and TGFβ1, thereby releasing the mature TGFβ1 for receptor binding. Active TGFβ1 binds to a type II receptor that recruits and phosphorylates type I receptor. The type I receptor then phosphorylates receptor-regulated SMADs (SMAD2 and SMAD3), which can bind the common mediator SMAD4 and subsequently translocate into the nucleus. In the nucleus, these SMADs interact with other transcription factors to upregulate the expression of target genes such as *SERPINE1* (PAI-1). SB431542 is a TGFβ1 receptor-1 kinase inhibitor.

Rapid activation of p38 MAPK by TGFβ1 was observed in luteal ECs; however, this pathway did not modulate the proapoptotic effect of TGFβ1 (Farberov & Meidan 2016). THBS1 can also activate the p38 pathway in these cells, but with slower kinetics than TGFβ1 (Farberov & Meidan 2016), indicating that this may be an indirect effect. Inhibition of the p38 pathway augmented the proapoptotic actions of THBS1 (Farberov & Meidan 2016). Activation of p38 kinase enhances cell survival in response to DNA damage, possibly by promoting DNA repair prior to a mitotic catastrophe (Thornton & Rincon 2009). Thus, this pathway may be initiated as a protective mechanism against DNA damage induced by proapoptotic THBS1.

TGFβ1 was found to induce the expression of *THBS1* in luteal ECs (Farberov & Meidan 2016) (Fig. 4) as well as in other cell types (Negoesu *et al.* 1995, Bein *et al.* 2004, Flugel-Koch *et al.* 2004). In addition to THBS1 stimulation, TGFβ1 also upregulates its own mRNA levels in these cells (Fig. 3) (Farberov & Meidan 2016), thus creating a positive feedback loop that leads to amplification of proapoptotic and antiangiogenic effects during luteolysis.

Role of THBS1 in matrix remodeling

Regulation of multiple proteolytic enzymes by THBS1 contributes to physiological and pathological tissue remodeling (Sweetwyne & Murphy-Ullrich 2012). Two interrelated proteolytic systems are involved in matrix remodeling, the plasminogen activation (PA) system and the matrix metalloproteinase (MMP) system. Several studies have shown that THBS1 is involved in regulating the balance between MMPs and MMP inhibitors (tissue inhibitors of metalloproteinases (TIMPs)). THBS1 prevents

the activation of MMPs via type I repeats (Bein & Simons 2000) and suppresses the activation of MMP9 by binding to its inactive form (Rodriguez-Manzanique *et al.* 2001). In HUVEC cells, inhibition of MMP activation decreased proangiogenic and promigratory effects induced by platelet microvesicles (Sun *et al.* 2017). On the other hand, THBS1 has also been reported to increase MMP2 and -9 activation in several tumor cells (Ren *et al.* 2006) and vascular smooth muscle cells (Lee *et al.* 2003). This dual behavior of THBS1 is probably mediated by distinct domains with opposite functions. These domains may also selectively activate or suppress the invasive behavior of vascular endothelium (Taraboletti *et al.* 2000).

THBS1 is also able to regulate the PA system. Upregulation of the *SERPINE1* gene product plasminogen activator inhibitor type 1 (PAI-1) by THBS1 has been demonstrated in several tumor cells (Albo *et al.* 1994, 1999), microvascular ECs (Bagavandoss *et al.* 1993), and recently in ECs derived from the CL (Farberov & Meidan 2016). PAI-1 is the primary inhibitor of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Inhibition of uPA/tPA blocks plasminogen-to-plasmin conversion as well as plasmin-dependent MMP activation (Pepper 2001). Additionally, PAI-1 can bind to vitronectin (VN), which leads to cell detachment and apoptosis (Chen *et al.* 2008).

Extensive tissue remodeling takes place in the ovary during follicular development, follicular rupture, CL formation and CL regression (Smith *et al.* 2002). Both MMP and PA systems have been implicated in CL formation and regression (Kliem *et al.* 2007). *MMP* mRNA expression was significantly increased in bovine CL during PGF2a-induced luteolysis, whereas *TIMP2* was decreased (Kliem *et al.* 2007). As mentioned, PGF2a-induced luteolysis also affects PAI-1 expression

Figure 5 Regulation of miR-221 expression and its role in luteal cells. miR-221 is highly expressed in luteal cells and in CL, where it effectively suppresses antiangiogenic THBS1. TGFβ1 and FGF2 inversely modulate miR-221 levels, providing a possible explanation for their reciprocal control of THBS1 expression. Consistent with THBS1 inhibition and consequent *SERPINE1* downregulation, miR-221 induces luteal endothelial cell migration, proliferation and survival, thereby promoting angiogenesis.

in a pattern resembling that of *THBS1*, a small and transient elevation during early CL formation and a substantial cumulative induction in regressing CL (Kliem *et al.* 2007, Farberov & Meidan 2016). Functionally, PAI-1 inhibits luteal EC–VN interactions either by the VN-binding domain or through antiprotease activity (Farberov & Meidan 2018), thereby affecting cell matrix adhesion required for cell survival.

These results are consistent with the following molecular cascade of events in bovine CL during PGF2a-induced luteolysis. (i) *THBS1* and *TGFβ1* are directly induced by PGF2a in luteal cells. (ii) *THBS1* then activates latent *TGFβ1*, leading to upregulation of *SERPINE1* (Figs 3 and 4). *THBS1*, *TGFβ1* and PAI-1 are expected to promote vascular instability as well as apoptosis and matrix remodeling in regressing CL.

Regulation of THBS1 expression by microRNAs

In addition to transcriptional regulation by FGF2 and TGFβ1, several studies have identified a number of miRNAs that can modulate THBS1 in different cell types (Vincenti *et al.* 2011, Dogar *et al.* 2014, Feng *et al.* 2015). Studies have suggested that miRNAs are critical for ovarian function by regulating folliculogenesis, oocyte–cumulus cell interaction, GC function and the development, maintenance and regression of the CL (Hossain *et al.* 2009, Christenson 2010, Maalouf *et al.* 2016). Recently, miR-221 was identified as a key regulator of THBS1 expression and function in ECs derived from bovine CL (Farberov & Meidan 2018). This miRNA, highly expressed in luteal cells and bovine CL, effectively reduced THBS1 levels and subsequently

inhibited *SERPINE1* mRNA (Farberov & Meidan 2018). The miR-221/222 cluster plays an important role in vascular biology, and miR-221 is among the most frequently upregulated miRNAs in human cancers (Vincenti *et al.* 2011, Nicoli *et al.* 2012, Lupini *et al.* 2013). Overexpression of miR-221 in luteal ECs was proangiogenic, consistent with THBS1 suppression (Fig. 5) (Farberov & Meidan 2018). Importantly, FGF2 and TGFβ1 were found to reciprocally regulate miR-221 in these cells. FGF2 elevates miR-221 levels, which may account for negative regulation of *THBS1* (Fig. 5). Conversely, TGFβ1, which stimulates *THBS1*, significantly reduces miR-221 levels (Fig. 5). Furthermore, FGF2 enhances the suppression of *THBS1* caused by miR-221 mimic and prevents the increase in *THBS1* induced by miR-221 inhibitor (Farberov & Meidan 2018). Importantly, FGF2 treatment also elevates miR-221 in bovine theca cells (Robinson *et al.* 2018). In contrast to FGF2, TGFβ1 reverses the inhibitory effect of miR-221 mimic on *THBS1* and enhances the upregulation of *THBS1* induced by miR-221 inhibitor (Farberov & Meidan 2018). These data support the contention that FGF2 and TGFβ1 modulate THBS1 expression via miR-221 (Fig. 5).

Figure 6 Illustrative summary of thrombospondin-1 (THBS1) actions in luteolysis. THBS1 and latent TGFβ1 are directly induced by PGF2a in mature CL. THBS1, a multimodular protein, exerts its antiangiogenic activity through multiple mechanisms: 1. apoptosis by activation of caspase-3; 2. interactions with two other vital luteal factors, FGF2 and TGFβ; 3. suppression of FGF2 expression and its angiogenic activities; 4. activation of latent TGFβ1, thus enabling TGFβ1 signaling, resulted in upregulation of *SERPINE1* (PAI-1). Disrupting the interaction between the uPA–uPA receptor complex and vitronectin by PAI-1 interrupts cell adhesion, further promoting cell death. Together, these actions of THBS1 contribute to a cascade of events leading to the irreversible demise of the CL.

Based on these findings, miR-221 is expected to be elevated during CL formation and reduced in late and regressed CL. Indeed, small RNA next-generation sequencing demonstrated that miR-221 levels vary in CL during the estrous cycle, with lower expression in late and regressed bovine CL and abundant expression in early CL (Gecaj *et al.* 2017). This temporal expression profile of miR-221 in CL is also supported by a study reporting that miR-221 expression in bovine plasma was lower on day 16 compared with day 8 of the estrous cycle (Ioannidis & Donadeu 2016). Similarly in sheep, greater miR-221 expression was found in early CL relatively to dominant follicle and reduced levels were found in late CL (McBride *et al.* 2012).

These findings suggest that miR-221 is regulated in a physiologically significant manner, affecting *THBS1* expression throughout the cycle. Altering *THBS1* levels can modulate vascular function in the CL, shifting from angiogenesis in young CL to vascular instability in late-cycle CL.

Conclusions and perspectives

We present evidence that *THBS1* is essential for luteal fate determination (regression or maintenance). Interactions with multiple membrane proteins, growth factors, extracellular matrix components and miRNAs regulated multiple pathways involved in luteal cell proliferation, migration and apoptosis (Fig. 6). Identifying the *THBS1* network of interactions with FGF2, TGF β 1 and SERPINE1 has enhanced our understanding of the molecular control of angiogenesis and cell survival in follicles and the CL. Crosstalk between pro- and antiangiogenic processes enables *THBS1* to inhibit angiogenesis by antagonizing survival pathways while also activating apoptotic pathways (Fig. 6). Recent investigations showing that miR-221 regulates *THBS1* expression in luteal cells provide new prospects for managing luteal cell fate and may facilitate the future development of effective and practical methods to fully regress the CL or optimize CL maintenance during early pregnancy. Further efforts are also needed to develop specific and potent therapeutic interventions that either downregulate or upregulate *THBS1* levels to promote or inhibit angiogenesis, which may prove useful in tackling angiogenesis-dependent diseases.

Declaration of interest

Rina Meidan is a member of the Editorial Board of *Reproduction*. Rina Meidan was not involved in the review or editorial process for this paper, on which she is listed as an author. The other authors have nothing to declare.

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References

- Adams J & Lawler J 1993 The thrombospondin family. *Current Biology* **3** 188–190. ([https://doi.org/10.1016/0960-9822\(93\)90270-X](https://doi.org/10.1016/0960-9822(93)90270-X))
- Adams JC & Lawler J 2004 The thrombospondins. *International Journal of Biochemistry and Cell Biology* **36** 961–968. (<https://doi.org/10.1016/j.biocel.2004.01.004>)
- Adams JC & Lawler J 2011 The thrombospondins. *Cold Spring Harbor Perspectives in Biology* **3** a009712. (<https://doi.org/10.1101/cshperspect.a009712>)
- Albo D, Arnoletti JP, Castiglioni A, Granick MS, Solomon MP, Rothman VL & Tuszyński GP 1994 Thrombospondin (TSP) and transforming growth factor beta 1 (TGF-beta) promote human A549 lung carcinoma cell plasminogen activator inhibitor type 1 (PAI-1) production and stimulate tumor cell attachment in vitro. *Biochemical and Biophysical Research Communications* **203** 857–865. (<https://doi.org/10.1006/bbrc.1994.2262>)
- Albo D, Berger DH, Vogel J & Tuszyński GP 1999 Thrombospondin-1 and transforming growth factor beta-1 upregulate plasminogen activator inhibitor type 1 in pancreatic cancer. *Journal of Gastrointestinal Surgery* **3** 411–417. ([https://doi.org/10.1016/S1091-255X\(99\)80058-4](https://doi.org/10.1016/S1091-255X(99)80058-4))
- Bagavandoss P, Kaytes P, Vogeli G, Wells PA & Wilks JW 1993 Recombinant truncated thrombospondin-1 monomer modulates endothelial cell plasminogen activator inhibitor 1 accumulation and proliferation in vitro. *Biochemical and Biophysical Research Communications* **192** 325–332. (<https://doi.org/10.1006/bbrc.1993.1418>)
- Bagavandoss P, Sage EH & Vernon RB 1998 Secreted protein, acidic and rich in cysteine (SPARC) and thrombospondin in the developing follicle and corpus luteum of the rat. *Journal of Histochemistry and Cytochemistry* **46** 1043–1049. (<https://doi.org/10.1177/002215549804600908>)
- Basavaraja R, Przygodzka E, Pawlinski B, Gajewski Z, Kaczmarek MM & Meidan R 2017 Interferon-tau promotes luteal endothelial cell survival and inhibits specific luteolytic genes in bovine corpus luteum. *Reproduction* **154** 559–568. (<https://doi.org/10.1530/REP-17-0290>)
- Bein K & Simons M 2000 Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *Journal of Biological Chemistry* **275** 32167–32173. (<https://doi.org/10.1074/jbc.M003834200>)
- Bein K, Odell-Fiddler ET & Drinane M 2004 Role of TGF-beta1 and JNK signaling in capillary tube patterning. *American Journal of Physiology: Cell Physiology* **287** C1012–C1022. (<https://doi.org/10.1152/ajpcell.00101.2004>)
- Berisha B, Schams D, Rodler D, Sinowatz F & Pfaffl MW 2016 Expression and localization of members of the thrombospondin family during final follicle maturation and corpus luteum formation and function in the bovine ovary. *Journal of Reproduction and Development* **62** 501–510. (<https://doi.org/10.1262/jrd.2016-056>)
- Boehm JR, Kutz SM, Sage EH, Staiano-Coico L & Higgins PJ 1999 Growth state-dependent regulation of plasminogen activator inhibitor type-1 gene expression during epithelial cell stimulation by serum and transforming growth factor- β 1. *Journal of Cellular Physiology* **181** 96–106. ([https://doi.org/10.1002/\(SICI\)1097-4652\(199910\)181:1<96::AID-JCP10>3.0.CO;2-I](https://doi.org/10.1002/(SICI)1097-4652(199910)181:1<96::AID-JCP10>3.0.CO;2-I))
- Bornstein P 2001 Thrombospondins as matricellular modulators of cell function. *Journal of Clinical Investigation* **107** 929–934. (<https://doi.org/10.1172/JCI2749>)
- Bornstein P 2009 Thrombospondins function as regulators of angiogenesis. *Journal of Cell Communication and Signaling* **3** 189–200. (<https://doi.org/10.1007/s12079-009-0060-8>)
- Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV *et al.* 2010 Uterine vein infusion of interferon tau (IFNT) extends luteal

- life span in ewes. *Biology of Reproduction* **82** 725–735. (<https://doi.org/10.1095/biolreprod.109.079467>)
- Brown EJ & Frazier WA 2001 Integrin-associated protein (CD47) and its ligands. *Trends in Cell Biology* **11** 130–135. ([https://doi.org/10.1016/S0962-8924\(00\)01906-1](https://doi.org/10.1016/S0962-8924(00)01906-1))
- Bruehl A, Touhami-Carrier M, Thomaidis A & Legrand C 2005 The proangiogenic phenotype of human tumor-derived endothelial cells depends on thrombospondin-1 downregulation via phosphatidylinositol 3-kinase/Akt pathway. *Journal of Molecular Medicine* **84** 852–863. (<https://doi.org/10.1007/s00109-006-0075-z>)
- Calzada MJ, Annis DS, Zeng B, Marcinkiewicz C, Banas B, Lawler J, Mosher DF & Roberts DD 2004 Identification of novel beta1 integrin binding sites in the type 1 and type 2 repeats of thrombospondin-1. *Journal of Biological Chemistry* **279** 41734–41743. (<https://doi.org/10.1074/jbc.M406267200>)
- Carlson CB, Lawler J & Mosher DF 2008 Structures of thrombospondins. *Cellular and Molecular Life Sciences* **65** 672–686. (<https://doi.org/10.1007/s00018-007-7484-1>)
- Chandrasekaran L, He C-Z, Al-Barazi H, Krutzsch HC, Iruela-Arispe ML & Roberts DD 2000 Cell contact-dependent activation of $\alpha\beta 1$ integrin modulates endothelial cell responses to thrombospondin-1. *Molecular Biology of the Cell* **11** 2885–2900. (<https://doi.org/10.1091/mbc.11.9.2885>)
- Chen H, Herndon ME & Lawler J 2000 The cell biology of thrombospondin-1. *Matrix Biology* **19** 597–614. ([https://doi.org/10.1016/S0945-053X\(00\)00107-4](https://doi.org/10.1016/S0945-053X(00)00107-4))
- Chen SC, Henry DO, Reczek PR & Wong MK 2008 Plasminogen activator inhibitor-1 inhibits prostate tumor growth through endothelial apoptosis. *Molecular Cancer Therapeutics* **7** 1227–1236. (<https://doi.org/10.1158/1535-7163.MCT-08-0051>)
- Christenson LK 2010 MicroRNA control of ovarian function. *Animal Reproduction* **7** 129–133.
- Colombo G, Margosio B, Ragona L, Neves M, Bonifacio S, Annis DS, Stravalaci M, Tomaselli S, Giavazzi R, Rusnati M *et al.* 2010 Non-peptidic thrombospondin-1 mimics as fibroblast growth factor-2 inhibitors: an integrated strategy for the development of new antiangiogenic compounds. *Journal of Biological Chemistry* **285** 8733–8742. (<https://doi.org/10.1074/jbc.M109.085605>)
- Dailey L, Ambrosetti D, Mansukhani A & Basilico C 2005 Mechanisms underlying differential responses to FGF signaling. *Cytokine and Growth Factor Reviews* **16** 233–247. (<https://doi.org/10.1016/j.cytogfr.2005.01.007>)
- Dogar AM, Semplicio G, Guenewig B & Hall J 2014 Multiple microRNAs derived from chemically synthesized precursors regulate thrombospondin 1 expression. *Nucleic Acid Therapeutics* **24** 149–159. (<https://doi.org/10.1089/nat.2013.0467>)
- Dreyfus M, Dardik R, Suh BS, Amsterdam A & Lahav J 1992 Differentiation-controlled synthesis and binding of thrombospondin to granulosa cells. *Endocrinology* **130** 2565–2570. (<https://doi.org/10.1210/endo.130.5.1374008>)
- Farberov S & Meidan R 2014 Functions and transcriptional regulation of thrombospondins and their interrelationship with fibroblast growth factor-2 in bovine luteal cells. *Biology of Reproduction* **91** 58. (<https://doi.org/10.1095/biolreprod.114.121020>)
- Farberov S & Meidan R 2016 Thrombospondin-1 affects bovine luteal function via transforming growth factor-beta1-dependent and independent actions. *Biology of Reproduction* **94** 25. (<https://doi.org/10.1095/biolreprod.115.135822>)
- Farberov S & Meidan R 2018 Fibroblast growth factor-2 and transforming growth factor-beta1 oppositely regulate miR-221 that targets thrombospondin-1 in bovine luteal endothelial cells. *Biology of Reproduction* **98** 366–375. (<https://doi.org/10.1093/biolre/iox167>)
- Feng N, Wang Z, Zhang Z, He X, Wang C & Zhang L 2015 miR-487b promotes human umbilical vein endothelial cell proliferation, migration, invasion and tube formation through regulating THBS1. *Neuroscience Letters* **591** 1–7. (<https://doi.org/10.1016/j.neulet.2015.02.002>)
- Flugel-Koch C, Ohlmann A, Fuchshofer R, Welge-Lüssen U & Tamm ER 2004 Thrombospondin-1 in the trabecular meshwork: localization in normal and glaucomatous eyes, and induction by TGF-beta1 and dexamethasone in vitro. *Experimental Eye Research* **79** 649–663. (<https://doi.org/10.1016/j.exer.2004.07.005>)
- Freyberg MA, Kaiser D, Graf R, Vischer P & Friedl P 2000 Integrin-associated protein and thrombospondin-1 as endothelial mechanosensitive death mediators. *Biochemical and Biophysical Research Communications* **271** 584–588. (<https://doi.org/10.1006/bbrc.2000.2678>)
- Garside SA, Harlow CR, Hillier SG, Fraser HM & Thomas FH 2010a Thrombospondin-1 inhibits angiogenesis and promotes follicular atresia in a novel in vitro angiogenesis assay. *Endocrinology* **151** 1280–1289. (<https://doi.org/10.1210/en.2009.0686>)
- Garside SA, Henkin J, Morris KD, Norvell SM, Thomas FH & Fraser HM 2010b A thrombospondin-mimetic peptide, ABT-898, suppresses angiogenesis and promotes follicular atresia in pre- and early-antral follicles in vivo. *Endocrinology* **151** 5905–5915. (<https://doi.org/10.1210/en.2010-0283>)
- Gecaj RM, Schanzenbach CI, Kirchner B, Pfaffl MW, Riedmaier I, Tweedie-Cullen RY & Berisha B 2017 The dynamics of microRNA transcriptome in bovine corpus luteum during its formation, function, and regression. *Frontiers in Genetics* **8** 213. (<https://doi.org/10.3389/fgene.2017.00213>)
- Greenaway J, Gentry PA, Feige JJ, LaMarre J & Petrik JJ 2005 Thrombospondin and vascular endothelial growth factor are cyclically expressed in an inverse pattern during bovine ovarian follicle development. *Biology of Reproduction* **72** 1071–1078. (<https://doi.org/10.1095/biolreprod.104.031120>)
- Hasan S, Hosseini G, Princivale M, Dong JC, Birsan D, Cagide C & de Agostini AI 2002 Coordinate expression of anticoagulant heparan sulfate proteoglycans and serine protease inhibitors in the rat ovary: a potent system of proteolysis control. *Biology of Reproduction* **66** 144–158. (<https://doi.org/10.1095/biolreprod66.1.144>)
- Higuchi T, Fujiwara H, Yamada S, Tatsumi K, Kataoka N, Itoh K, Maeda M, Fujita J & Fujii S 1999 Co-expression of integrin-associated protein (IAP/CD47) and its ligand thrombospondin-1 on human granulosa and large luteal cells. *Molecular Human Reproduction* **5** 920–926. (<https://doi.org/10.1093/molehr/5.10.920>)
- Hossain MM, Ghanem N, Hoelker M, Rings F, Phatsara C, Tholen E, Schellander K & Tesfaye D 2009 Identification and characterization of miRNAs expressed in the bovine ovary. *BMC Genomics* **10** 443. (<https://doi.org/10.1186/1471-2164-10-443>)
- Ioannidis J & Donadeu FX 2016 Circulating microRNA profiles during the bovine oestrous cycle. *PLoS ONE* **11** e0158160. (<https://doi.org/10.1371/journal.pone.0158160>)
- Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL & Bouck N 2000 Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nature Medicine* **6** 41–48. (<https://doi.org/10.1038/71517>)
- Juengel JL, Garverick HA, Johnson AL, Youngquist RS & Smith MF 1993 Apoptosis during luteal regression in cattle. *Endocrinology* **132** 249–254. (<https://doi.org/10.1210/endo.132.1.8419126>)
- Kang JH, Chang SY, Yeom DH, Kim SA, Um SJ & Hong KJ 2004 Weakening of the repressive YY-1 site on the thrombospondin-1 promoter via c-Jun/YY-1 interaction. *Experimental and Molecular Medicine* **36** 300–310. (<https://doi.org/10.1038/emmm.2004.41>)
- Kim M-S, Oh YJ, Lee S, Kim JE, Kim KH & Chung JH 2006 Ultraviolet radiation attenuates thrombospondin 1 expression via PI3K-Akt activation in human keratinocytes. *Photochemistry and Photobiology* **82** 645–650. (<https://doi.org/10.1562/2005-09-29-RA-702>)
- Kliem H, Welter H, Kraetzel WD, Steffl M, Meyer HH, Schams D & Berisha B 2007 Expression and localisation of extracellular matrix degrading proteases and their inhibitors during the oestrous cycle and after induced luteolysis in the bovine corpus luteum. *Reproduction* **134** 535–547. (<https://doi.org/10.1530/REP-06-0172>)
- Kvansakul M, Adams JC & Hohenester E 2004 Structure of a thrombospondin C-terminal fragment reveals a novel calcium core in the type 3 repeats. *EMBO Journal* **23** 1223–1233. (<https://doi.org/10.1038/sj.emboj.7600166>)
- Kyriakides TR, Zhu Y-H, Smith LT, Bain SD, Yang Z, Lin MT, Danielson KG, Iozzo RV, LaMarca M & McKinney CE 1998 Mice that lack thrombospondin 2 display connective tissue abnormalities that are associated with disordered collagen fibrillogenesis, an increased vascular density, and a bleeding diathesis. *Journal of Cell Biology* **140** 419–430. (<https://doi.org/10.1083/jcb.140.2.419>)

- Lauderdale JW 2009 ASAS centennial paper: contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. *Journal of Animal Science* **87** 801–812. (<https://doi.org/10.2527/jas.2008-1407>)
- Lawler J 2000 The functions of thrombospondin-1 and-2. *Current Opinion in Cell Biology* **12** 634–640. ([https://doi.org/10.1016/S0955-0674\(00\)00143-5](https://doi.org/10.1016/S0955-0674(00)00143-5))
- Lawler J, Sunday M, Thibert V, Duquette M, George EL, Rayburn H & Hynes RO 1998 Thrombospondin-1 is required for normal murine pulmonary homeostasis and its absence causes pneumonia. *Journal of Clinical Investigation* **101** 982–992. (<https://doi.org/10.1172/JCI1684>)
- Lawler J, Miao WM, Duquette M, Bouck N, Bronson RT & Hynes RO 2001 Thrombospondin-1 gene expression affects survival and tumor spectrum of p53-deficient mice. *American Journal of Pathology* **159** 1949–1956. ([https://doi.org/10.1016/S0002-9440\(10\)63042-8](https://doi.org/10.1016/S0002-9440(10)63042-8))
- Lee T, Esemuede N, Sumpio BE & Gahtan V 2003 Thrombospondin-1 induces matrix metalloproteinase-2 activation in vascular smooth muscle cells. *Journal of Vascular Surgery* **38** 147–154. ([https://doi.org/10.1016/S0741-5214\(02\)75468-2](https://doi.org/10.1016/S0741-5214(02)75468-2))
- Liebermann J, Schams D & Miyamoto A 1996 Effects of local growth factors on the secretory function of bovine corpus luteum during the oestrous cycle and pregnancy in vitro. *Reproduction, Fertility and Development* **8** 1003–1011. (<https://doi.org/10.1071/RD9961003>)
- Lupini L, Bassi C, Ferracin M, Bartonicek N, D'Abundo L, Zagatti B, Callegari E, Musa G, Moshiri F, Gramantieri L *et al.* 2013 miR-221 affects multiple cancer pathways by modulating the level of hundreds messenger RNAs. *Frontiers in Genetics* **4** 64. (<https://doi.org/10.3389/fgene.2013.00064>)
- Maalouf SW, Smith CL & Pate JL 2016 Changes in microRNA expression during maturation of the bovine corpus luteum: regulation of luteal cell proliferation and function by microRNA-34a. *Biology of Reproduction* **94** 71. (<https://doi.org/10.1095/biolreprod.115.135053>)
- Maroni D & Davis JS 2011 TGF β 1 disrupts the angiogenic potential of microvascular endothelial cells of the corpus luteum. *Journal of Cell Science* **124** 2501–2510. (<https://doi.org/10.1242/jcs.084558>)
- Massague J 2000 How cells read TGF- β signals. *Nature Reviews Molecular Cell Biology* **1** 169–178. (<https://doi.org/10.1038/35043051>)
- Mattila MM, Tarkkonen KM, Seppanen JA, Ruohola JK, Valve EM & Harkonen PL 2006 Androgen and fibroblast growth factor 8 (FGF8) downregulation of thrombospondin 1 (TSP1) in mouse breast cancer cells. *Molecular and Cellular Endocrinology* **253** 36–43. (<https://doi.org/10.1016/j.mce.2006.04.007>)
- McBride D, Carre W, Sontakke SD, Hogg CO, Law A, Donadeu FX & Clinton M 2012 Identification of miRNAs associated with the follicular-luteal transition in the ruminant ovary. *Reproduction* **144** 221–233. (<https://doi.org/10.1530/REP-12-0025>)
- McCracken JA, Custer EE & Lamsa JC 1999 Luteolysis: a neuroendocrine-mediated event. *Physiological Reviews* **79** 263–323. (<https://doi.org/10.1152/physrev.1999.79.2.263>)
- Meidan R, Girsch E, Mamluk R, Levy N & Farberov S 2017 *Luteolysis in Ruminants: Past Concepts, New Insights, and Persisting Challenges, The Life Cycle of the Corpus Luteum*, pp 159–182. Springer International Publishing: Switzerland.
- Miyamoto A, Shirasuna K, Shimizu T, Bollwein H & Schams D 2010 Regulation of corpus luteum development and maintenance: specific roles of angiogenesis and action of prostaglandin F $_{2\alpha}$. *Society of Reproduction and Fertility Supplement* **67** 289–304.
- Mondal M, Schilling B, Folger J, Steibel JP, Buchnick H, Zalman Y, Ireland JJ, Meidan R & Smith GW 2011 Deciphering the luteal transcriptome: potential mechanisms mediating stage-specific luteolytic response of the corpus luteum to prostaglandin F $_{2\alpha}$. *Physiological Genomics* **43** 447–456. (<https://doi.org/10.1152/physiolgenomics.00155.2010>)
- Murphy-Ullrich JE & Poczatek M 2000 Activation of latent TGF- β by thrombospondin-1: mechanisms and physiology. *Cytokine and Growth Factor Reviews* **11** 59–69. ([https://doi.org/10.1016/S1359-6101\(99\)00029-5](https://doi.org/10.1016/S1359-6101(99)00029-5))
- Negoesu A, Lafeyllade B, Pellerin S, Chambaz EM & Feige JJ 1995 Transforming growth factors beta stimulate both thrombospondin-1 and CISP/thrombospondin-2 synthesis by bovine adrenocortical cells. *Experimental Cell Research* **217** 404–409. (<https://doi.org/10.1006/excr.1995.1103>)
- Nicholson AC, Han J, Febbraio M, Silverstein RL & Hajjar DP 2001 Role of CD36, the macrophage class B scavenger receptor, in atherosclerosis. *Annals of the New York Academy of Sciences* **947** 224–228. (<https://doi.org/10.1111/j.1749-6632.2001.tb03944.x>)
- Nicoli S, Knyphausen CP, Zhu LJ, Lakshmanan A & Lawson ND 2012 miR-221 is required for endothelial tip cell behaviors during vascular development. *Developmental Cell* **22** 418–429. (<https://doi.org/10.1016/j.devcel.2012.01.008>)
- Niswender GD, Juengel JL, Silva PJ, Rollyson MK & McIntush EW 2000 Mechanisms controlling the function and life span of the corpus luteum. *Physiological Reviews* **80** 1–29. (<https://doi.org/10.1152/physrev.2000.80.1.1>)
- Pagano K, Torella R, Foglieni C, Bugatti A, Tomaselli S, Zetta L, Presta M, Rusnati M, Tarabozetti G, Colombo G *et al.* 2012 Direct and allosteric inhibition of the FGF2/HSPGs/FGFR1 ternary complex formation by an antiangiogenic, thrombospondin-1-mimic small molecule. *PLoS ONE* **7** e36990. (<https://doi.org/10.1371/journal.pone.0036990>)
- Passiatore G, Gentilella A, Rom S, Pacifici M, Bergonzini V & Peruzzi F 2011 Induction of Id-1 by FGF-2 involves activity of EGR-1 and sensitizes neuroblastoma cells to cell death. *Journal of Cellular Physiology* **226** 1763–1770. (<https://doi.org/10.1002/jcp.22505>)
- Pepper MS 2001 Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. *Arteriosclerosis, Thrombosis and Vascular Biology* **21** 1104–1117. (<https://doi.org/10.1161/hq0701.093685>)
- Petrik JJ, Gentry PA, Feige JJ & LaMarre J 2002 Expression and localization of thrombospondin-1 and -2 and their cell-surface receptor, CD36, during rat follicular development and formation of the corpus luteum. *Biology of Reproduction* **67** 1522–1531. (<https://doi.org/10.1095/biolreprod.102.007153>)
- Ren B, Yee KO, Lawler J & Khosravi-Far R 2006 Regulation of tumor angiogenesis by thrombospondin-1. *Biochimica et Biophysica Acta (BBA): Reviews on Cancer* **1765** 178–188. (<https://doi.org/10.1016/j.bbcan.2005.11.002>)
- Resovi A, Pinessi D, Chiorino G & Tarabozetti G 2014 Current understanding of the thrombospondin-1 interactome. *Matrix Biology* **37** 83–91. (<https://doi.org/10.1016/j.matbio.2014.01.012>)
- Ribeiro SM, Poczatek M, Schultz-Cherry S, Villain M & Murphy-Ullrich JE 1999 The activation sequence of thrombospondin-1 interacts with the latency-associated peptide to regulate activation of latent transforming growth factor- β . *Journal of Biological Chemistry* **274** 13586–13593. (<https://doi.org/10.1074/jbc.274.19.13586>)
- Robertson IB & Rifkin DB 2013 Unchaining the beast: insights from structural and evolutionary studies on TGF β secretion, sequestration, and activation. *Cytokine and Growth Factor Reviews* **24** 355–372. (<https://doi.org/10.1016/j.cytogfr.2013.06.003>)
- Robinson R, Nicklin L, Hammond A, Schams D, Hunter M & Mann G 2007 Fibroblast growth factor 2 is more dynamic than vascular endothelial growth factor A during the follicle-luteal transition in the cow. *Biology of Reproduction* **77** 28–36. (<https://doi.org/10.1095/biolreprod.106.055434>)
- Robinson CL, Zhang L, Schutz LF, Totty ML & Spicer LJ 2018 MicroRNA 221 expression in theca and granulosa cells: hormonal regulation and function. *Journal of Animal Science* **96** 641–652. (<https://doi.org/10.1093/jas/skx069>)
- Rodriguez-Manzanique JC, Lane TF, Ortega MA, Hynes RO, Lawler J & Iruela-Arispe ML 2001 Thrombospondin-1 suppresses spontaneous tumor growth and inhibits activation of matrix metalloproteinase-9 and mobilization of vascular endothelial growth factor. *PNAS* **98** 12485–12490. (<https://doi.org/10.1073/pnas.171460498>)
- Romero JJ, Antoniazzi AQ, Smirnova NP, Webb BT, Yu F, Davis JS & Hansen TR 2013 Pregnancy-associated genes contribute to antiluteolytic mechanisms in ovine corpus luteum. *Physiological Genomics* **45** 1095–1108. (<https://doi.org/10.1152/physiolgenomics.00082.2013>)
- Romero JJ, Antoniazzi AQ, Nett TM, Ashley RL, Webb BT, Smirnova NP, Bott RC, Bruemmer JE, Bazer FW, Anthony RV *et al.* 2015 Temporal release, paracrine and endocrine actions of ovine conceptus-derived interferon-tau during early pregnancy. *Biology of Reproduction* **93** 146. (<https://doi.org/10.1095/biolreprod.115.132860>)
- Rueda BR, Hendry IR, Tilly JL & Hamernik DL 1999 Accumulation of caspase-3 messenger ribonucleic acid and induction of caspase activity in the ovine corpus luteum following prostaglandin F $_{2\alpha}$ treatment in vivo. *Biology of Reproduction* **60** 1087–1092. (<https://doi.org/10.1095/biolreprod60.5.1087>)

- Sanchez-Capelo A 2005 Dual role for TGF-beta1 in apoptosis. *Cytokine and Growth Factor Reviews* **16** 15–34. (<https://doi.org/10.1016/j.cytogfr.2004.11.002>)
- Santiago FS, Lowe HC, Bobryshev YV & Khachigian LM 2001 Induction of the transcriptional repressor yin yang-1 by vascular cell injury autocrine/paracrine role of endogenous fibroblast growth factor-2. *Journal of Biological Chemistry* **276** 41143–41149. (<https://doi.org/10.1074/jbc.M104913200>)
- Schams D & Berisha B 2004 Regulation of corpus luteum function in cattle – an overview. *Reproduction in Domestic Animals* **39** 241–251. (<https://doi.org/10.1111/j.1439-0531.2004.00509.x>)
- Shirasuna K, Nitta A, Sineenard J, Shimizu T, Bollwein H & Miyamoto A 2012 Vascular and immune regulation of corpus luteum development, maintenance, and regression in the cow. *Domestic Animal Endocrinology* **43** 198–211. (<https://doi.org/10.1016/j.domaniend.2012.03.007>)
- Simantov R, Febbraio M & Silverstein R 2005 The antiangiogenic effect of thrombospondin-2 is mediated by CD36 and modulated by histidine-rich glycoprotein. *Matrix Biology* **24** 27–34. (<https://doi.org/10.1016/j.matbio.2004.11.005>)
- Smith MF, Ricke WA, Bakke LJ, Dow MP & Smith GW 2002 Ovarian tissue remodeling: role of matrix metalloproteinases and their inhibitors. *Molecular and Cellular Endocrinology* **191** 45–56. ([https://doi.org/10.1016/S0303-7207\(02\)00054-0](https://doi.org/10.1016/S0303-7207(02)00054-0))
- Sottile J, Selegue J & Mosher DF 1991 Synthesis of truncated amino-terminal trimers of thrombospondin. *Biochemistry* **30** 6556–6562. (<https://doi.org/10.1021/bi00240a028>)
- Staniszewska I, Zaveri S, Del Valle L, Oliva I, Rothman VL, Croul SE, Roberts DD, Mosher DF, Tuszyński GP & Marcinkiewicz C 2007 Interaction of alpha9beta1 integrin with thrombospondin-1 promotes angiogenesis. *Circulation Research* **100** 1308–1316. (<https://doi.org/10.1161/01.RES.0000266662.98355.66>)
- Sun C, Feng SB, Cao ZW, Bei JJ, Chen Q, Zhao WB, Xu XJ, Zhou Z, Yu ZP & Hu HY 2017 Up-regulated expression of matrix metalloproteinases in endothelial cells mediates platelet microvesicle-induced angiogenesis. *Cellular Physiology and Biochemistry* **41** 2319–2332. (<https://doi.org/10.1159/000475651>)
- Sweetwyne MT & Murphy-Ullrich JE 2012 Thrombospondin 1 in tissue repair and fibrosis: TGF-beta-dependent and independent mechanisms. *Matrix Biology* **31** 178–186. (<https://doi.org/10.1016/j.matbio.2012.01.006>)
- Tan K, Duquette M, Liu JH, Zhang R, Joachimiak A, Wang JH & Lawler J 2006 The structures of the thrombospondin-1 N-terminal domain and its complex with a synthetic pentameric heparin. *Structure* **14** 33–42. (<https://doi.org/10.1016/j.str.2005.09.017>)
- Taraboletti G, Morbidelli L, Donnini S, Parenti A, Granger HJ, Giavazzi R & Ziche M 2000 The heparin binding 25 kDa fragment of thrombospondin-1 promotes angiogenesis and modulates gelatinase and TIMP-2 production in endothelial cells. *FASEB Journal* **14** 1674–1676. (<https://doi.org/10.1096/fj.99-0931fje>)
- Thomas FH, Wilson H, Silvestri A & Fraser HM 2008 Thrombospondin-1 expression is increased during follicular atresia in the primate ovary. *Endocrinology* **149** 185–192. (<https://doi.org/10.1210/en.2007-0835>)
- Thornton TM & Rincon M 2009 Non-classical p38 map kinase functions: cell cycle checkpoints and survival. *International Journal of Biological Sciences* **5** 44–51. (<https://doi.org/10.7150/ijbs.5.44>)
- Tsang M & Dawid IB 2004 Promotion and attenuation of FGF signaling through the Ras-MAPK pathway. *Science Signaling* **2004** pe17. (<https://doi.org/10.1126/stke.2282004pe17>)
- Vincenti S, Brillante N, Lanza V, Bozzoni I, Presutti C, Chiani F, Etna MP & Negri R 2011 HUVEC respond to radiation by inducing the expression of pro-angiogenic microRNAs. *Radiation Research* **175** 535–546. (<https://doi.org/10.1667/RR2200.1>)
- Vogel T, Guo NH, Kruttsch HC, Blake DA, Hartman J, Mendelovitz S, Panet A & Roberts DD 1993 Modulation of endothelial cell proliferation, adhesion, and motility by recombinant heparin-binding domain and synthetic peptides from the type I repeats of thrombospondin. *Journal of Cellular Biochemistry* **53** 74–84. (<https://doi.org/10.1002/jcb.240530109>)
- Volpert OV, Pili R, Sikder HA, Nelius T, Zaichuk T, Morris C, Shiflett CB, Devlin MK, Conant K & Alani RM 2002 Id1 regulates angiogenesis through transcriptional repression of thrombospondin-1. *Cancer Cell* **2** 473–483. ([https://doi.org/10.1016/S1535-6108\(02\)00209-X](https://doi.org/10.1016/S1535-6108(02)00209-X))
- Wang S, Wu Z, Sorenson CM, Lawler J & Sheibani N 2003 Thrombospondin-1-deficient mice exhibit increased vascular density during retinal vascular development and are less sensitive to hyperoxia-mediated vessel obliteration. *Developmental Dynamics* **228** 630–642. (<https://doi.org/10.1002/dvdy.10412>)
- Xing C, Lee S, Kim WJ, Jin G, Yang YG, Ji X, Wang X & Lo EH 2009 Role of oxidative stress and caspase 3 in CD47-mediated neuronal cell death. *Journal of Neurochemistry* **108** 430–436. (<https://doi.org/10.1111/j.1471-4159.2008.05777.x>)
- Yadav VK, Lakshmi G & Medhamurthy R 2005 Prostaglandin F2alpha-mediated activation of apoptotic signaling cascades in the corpus luteum during apoptosis: involvement of caspase-activated DNase. *Journal of Biological Chemistry* **280** 10357–10367. (<https://doi.org/10.1074/jbc.M409596200>)
- Yamashita H, Kamada D, Shirasuna K, Matsui M, Shimizu T, Kida K, Berisha B, Schams D & Miyamoto A 2008 Effect of local neutralization of basic fibroblast growth factor or vascular endothelial growth factor by a specific antibody on the development of the corpus luteum in the cow. *Molecular Reproduction and Development* **75** 1449–1456. (<https://doi.org/10.1002/mrd.20878>)
- Yanagishita M 1994 Proteoglycans and hyaluronan in female reproductive organs. *Exs* **70** 179–190.
- Zabuawala T, Taffany DA, Sharma SM, Merchant A, Adair B, Srinivasan R, Rosol TJ, Fernandez S, Huang K & Leone G 2010 An ets2-driven transcriptional program in tumor-associated macrophages promotes tumor metastasis. *Cancer Research* **70** 1323–1333. (<https://doi.org/10.1158/0008-5472.CAN-09-1474>)
- Zalman Y, Klipper E, Farberov S, Mondal M, Wee G, Folger JK, Smith GW & Meidan R 2012 Regulation of angiogenesis-related prostaglandin f2alpha-induced genes in the bovine corpus luteum. *Biology of Reproduction* **86** 92. (<https://doi.org/10.1095/biolreprod.111.095067>)
- Zhang YE 2009 Non-Smad pathways in TGF-beta signaling. *Cell Research* **19** 128–139. (<https://doi.org/10.1038/cr.2008.328>)

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