Mare endometrosis and pro-fibrotic cytokines: what's new?

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Transforming growth factor beta 1 (TGF-β1) and its receptors I and II (TGFRI, TGFRII), Platelet derived growth factor (PDGF), Connective tissue growth factor (CTGF), Matrix metalloproteinases (MMPs) and Tissue inhibitor of metalloproteinase 1 (TIMP-1) are important in fibrogenesis. Endometrosis is a mare endometrial disorder where tissue structure disruption and dysfunction result from excessive deposition of extracellular-matrix (ECM), like type I and III collagens (COL1, COL3). Our studies on follicular phase (FP) endometrium explants treated with TGF-β1, PDGF or CTGF upregulated COL1 and COL3 gene transcription at different incubation times. Thus, the aim was to evaluate TGF-β1, PDGF and CTGF *in vitro* effects on expression of genes COL1, COL3, TGF-β1, TGFRI, TGFRII, MMP-2, MMP-9, TIMP-1 and TGF-β1, TIMP-1, TNFα, PGE_2 and PGF2α secretion in mare luteal phase (LP) endometrium.

Endometrium explants (n=5) were cultured (24h, 48h, 72h) with TGF-β1 (1, 10ng/ml), PDGF (0.1, 5ng/ml), CTGF (100, 200ng/ml), TNFα (10ng/ml) or oxytocin (10^{-7} M). Real-time PCR was used for mRNA transcription evaluation. Protein expression was quantified in culture medium by Elisa.

TGF-β1 (10ng/ml) raised mRNA of COL1 (24h, 72h), COL3 (72h), TGFRI (24h, 72h), TGFRII (72h) ($P<0.05$) and MMP-9 (24h, $P<0.001$; 72h, $P<0.05$). TGF-β1 protein expression was up-regulated at all times ($p< 0.0001$). CTGF effect on COL1 and COL3 mRNA was dose and time dependent. At 48h, CTGF (100ng/ml) increased COL1 and TGFRI and decreased COL3 mRNA ($P<0.05$). At 72h, high dose CTGF raised COL1, COL3 and TGF-β1 mRNA transcription ($P<0.05$). CTGF (100ng/ml at 48h; 200ng/ml at 72h) decreased protein expression of PGE_2 ($P<0.05$), raised PGF2α/PGE_2 ratio ($P<0.001$) and TIMP-1 protein ($P<0.05$). TNFα up-regulated COL1, TGF-β1, TGFRI, TGFRII, MMP-9 mRNA transcription ($P<0.05$), at 48h. TNFα protein expression was up-regulated at all times ($p< 0.0001$).

In LP, the positive cross-talk between PDGF and TGF-β1 noticed in FP, was not detected. Besides TGF-β1 direct effect on its receptors, it directly induces MMP-9 expression. Since TGF-β1 is secreted in inactive form, MMP-9 may cleave latent TGF-β and activate it. CTGF appears to mediate endometrium fibrosis through stimulation of TGF-β1 signaling and TIMP-1 production. TIMP-1 might prevent MMPs ECM degradation and thus participate in ECM accumulation. Besides, a high PGF2α/PGE_2 ratio seems to generate an enabling cytokine environment to CTGF induced fibrosis. TNF may mediate endometrium fibrosis through TGF-β1 and MMP-9 actions. In conclusion, this study shows a possible involvement of TGF-β1, CTGF and TNF in the development of mare endometrial fibrosis during luteal phase.