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## The role of TGF- $\beta$ in the pathophysiology of peritoneal endometriosis

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35                  15                  **Running title:** Transforming growth factor in peritoneal endometriosis  
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1  
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3 **Abstract**  
4

5 **BACKGROUND:** Endometriosis is estimated to affect 6-10% of women of reproductive age  
6  
7 and it is associated with chronic pelvic pain, dysmenorrhea and subfertility. It is currently  
8  
9 managed surgically or medically but symptoms recur in up to 75% of cases and available  
10  
11 medical treatments have undesirable side effects. Endometriosis is defined as the presence of  
12  
13 endometrial tissue outside the uterus with lesions typically found on the peritoneum. The  
14  
15 aetiology of endometriosis is uncertain but there is increasing evidence that transforming  
16  
17 growth factor (TGF)- $\beta$  plays a major role.  
18

19 **OBJECTIVE AND RATIONALE:** A descriptive review was undertaken of the published  
20  
21 literature on the expression pattern of TGF- $\beta$  ligands and signalling molecules in women with  
22  
23 and without endometriosis, and on the potential roles of TGF- $\beta$  signalling in the development  
24  
25 and progression of peritoneal endometriosis. The current understanding of the TGF-beta  
26  
27 signaling pathway is summarized.  
28

29 **SEARCH METHODS:** We searched the Pubmed database using the terms ‘transforming  
30  
31 growth factor beta’ and ‘endometriosis’ for studies published between 1995 and 2016. The  
32  
33 initial search identified 99 studies and these were used as the basic material for this review.  
34  
35 We also extended our remit for important older publications. In addition, we searched the  
36  
37 reference lists of studies used in this review for additional studies we judged as relevant.  
38  
39 Studies which were included in the review focused on peritoneal endometriosis only as  
40  
41 increasing evidence suggests that ovarian and deep endometriosis may have a differing  
42  
43 pathophysiology. Thus, a final 95 studies were included in the review.  
44  
45

46 **OUTCOMES:** TGF- $\beta$ 1 is reported to be increased in the peritoneal fluid, serum, ectopic  
47  
48 endometrium and peritoneum of women with endometriosis compared to women without  
49  
50 endometriosis, and TGF- $\beta$ 1-null mice have reduced endometriosis lesion growth when  
51  
52 compared to their wild-type controls. Studies in mice and women have indicated that  
53  
54 increasing levels of TGF- $\beta$  ligands are associated with decreased immune cell activity within  
55  
56 the peritoneum, together with an increase in ectopic endometrial cell survival, attachment,  
57  
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3 59 invasion and proliferation, during endometriosis lesion development. TGF- $\beta$ 1 has been  
4  
5 60 associated with changes in ectopic endometrial and peritoneal cell metabolism and the  
6  
7 61 initiation of neoangiogenesis, further fuelling endometriosis lesion development.  
8

9 62 **WIDER IMPLICATIONS:** Together these studies suggest that TGF- $\beta$ 1 plays a major role  
10  
11 63 in the development of peritoneal endometriosis lesions and that targeting this pathway may be  
12  
13 64 of therapeutic potential.  
14

15 65

16 66 **Keywords**

17 67 endometriosis, endometrium, peritoneum, smad, immune cells, angiogenesis  
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For Peer Review

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3 **68 INTRODUCTION**

4  
5 69 Endometriosis is estimated to affect 6-10% of women of reproductive age and is associated  
6  
7 70 with chronic pelvic pain, dysmenorrhea, dyspareunia and subfertility (Meuleman et al. 2009;  
8  
9 71 Giudice & Kao 2004). These symptoms affect general physical, mental and social well-being  
10  
11 72 and have a significant impact on quality of life (Dunselman et al. 2014). Endometriosis is  
12  
13 73 currently diagnosed by laparoscopy, but the time to diagnosis can be long (on average 6-7  
14  
15 74 years) owing to the variability of the symptoms and a lack of diagnostic biomarkers  
16  
17 75 (Nnoaham et al. 2011). Symptoms can be managed medically or surgically but symptoms  
18  
19 76 reoccur in up to 75% of surgical cases within 2 years and available medical treatments have  
20  
21 77 undesirable side effects and are contraceptive (Jacobson et al. 2009). The annual average  
22  
23 78 health care cost associated with endometriosis in the UK is estimated at £8.5 billion, which is  
24  
25 79 similar to that of diabetes and rheumatoid arthritis (Simoens et al. 2012).

26  
27 80  
28  
29 81 Endometriosis is a benign, estrogen-dependent disorder defined as the presence of  
30  
31 82 endometrial glands and stroma outside the uterine cavity (Giudice 2010). It is now generally  
32  
33 83 accepted that there are three distinct types of endometriosis: peritoneal, ovarian and deep  
34  
35 84 endometriosis, each of which is thought to have a different pathogenesis (Nisolle & Donnez  
36  
37 85 1997). The most common type of endometriosis is peritoneal endometriosis and this is the  
38  
39 86 focus of our review (Mahmood & Templeton 1991).

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41 87  
42  
43 88 The widely accepted hypothesis for the development of endometriosis is the retrograde  
44  
45 89 menstruation theory proposed by Sampson in 1927. This theory suggests that during  
46  
47 90 menstruation viable endometrial tissue is refluxed through the Fallopian tubes into the  
48  
49 91 peritoneal cavity where it implants and grows (Sampson 1927). Sampson's theory is  
50  
51 92 supported by the high prevalence of pelvic endometriosis in girls with congenital menstrual  
52  
53 93 outflow obstruction and the distribution of lesions in the abdominal cavity (Nap 2004). It is  
54  
55 94 also supported by the fact that women with endometriosis have more frequent sub-  
56  
57 95 endometrial myometrial contractile waves than women without endometriosis (Salamanca &

1  
2  
3 96 Beltrán 1995) In addition women with endometriosis have higher volumes of refluxed  
4  
5 97 menstrual blood than healthy controls (Halme et al. 1984; Salamanca & Beltrán 1995).  
6  
7 98 However, as retrograde menstruation is seen in over 90% of women, this hypothesis fails to  
8  
9 99 fully explain why shed endometrial tissue implants in some women and not in others (Halme  
10  
11 100 et al. 1984).

12  
13 101

14  
15 102 It is now agreed that a combination of genetic, hormonal, immunological and anatomical  
16  
17 103 factors contribute to the formation and development of endometrial lesions (Giudice & Kao  
18  
19 104 2004). The formation of peritoneal lesions has been attributed to the attachment of ectopic  
20  
21 105 endometrium to the peritoneal surface, invasion of the peritoneum, neoangiogenesis,  
22  
23 106 suppression of the immune system and continued survival and growth of lesion tissue  
24  
25 107 (Giudice & Kao 2004; Young et al, 2013). Increased concentrations of inflammatory  
26  
27 108 cytokines and growth factors within the peritoneal fluid and peritoneal tissue are thought to  
28  
29 109 contribute to peritoneal lesion formation (Young et al. 2013). Transforming growth factor  
30  
31 110 beta (TGF- $\beta$ ) is an inflammatory growth factor that regulates a variety of cellular functions  
32  
33 111 including cell adhesion, invasion and angiogenesis, all of which are essential during  
34  
35 112 endometriosis lesion development. Levels of TGF- $\beta$  are reported to be increased in the  
36  
37 113 peritoneal fluid, serum, ectopic endometrium and peritoneal tissue of women with  
38  
39 114 endometriosis compared to controls (Oosterlynck et al. 1994; Pizzo et al. 2002; Chegini et al.  
40  
41 115 1994; Young et al. 2014a; Young et al. 2014b) and *Tgfb1* null mice have reduced  
42  
43 116 endometriosis lesion growth when compared to wild-type controls (Hull et al. 2012),  
44  
45 117 suggesting TGF- $\beta$ 1 plays a key role in lesion development. Nevertheless, the functional role  
46  
47 118 that TGF- $\beta$  plays in the pathophysiology of endometriosis is less clear. This review will  
48  
49 119 attempt to highlight the expression pattern and potential roles of TGF- $\beta$  ligands and signalling  
50  
51 120 in the pathophysiology of peritoneal endometriosis.  
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## 122 **METHODS**

123 We searched the Pubmed database using the terms ‘transforming growth factor beta’ and  
124 ‘endometriosis’ for studies published between 1995 and 2016. The initial search identified 99  
125 studies and these were used as the basic material for this review. We also extended our remit  
126 for important older publications. In addition, we searched the reference lists of studies used in  
127 this review for additional studies we judged as relevant. Studies which were included in the  
128 review focused on peritoneal endometriosis only as increasing evidence suggests that ovarian  
129 and deep endometriosis may have a differing pathophysiology. Thus, a final 95 studies were  
130 included in the review.

## 131 **RESULTS**

### 132 **The TGF- $\beta$ signalling pathway**

133 The TGF- $\beta$  superfamily consists of over 30 different ligands in humans and includes three  
134 TGF- $\beta$  isoforms, four activin isoforms, 10 bone morphogenetic protein isoforms, 11 growth  
135 and differentiation factor isoforms and the protein nodal (Schmierer & Hill 2007). TGF- $\beta$  is  
136 secreted in a latent complex consisting of three proteins: TGF- $\beta$ , an inhibitor (latency-  
137 associated protein, LAP, which is derived from the TGF- $\beta$  propeptide) and an extracellular  
138 matrix (ECM)-binding protein (latent TGF- $\beta$  binding proteins, or LTBPs). LTBPs interact  
139 with fibrillins and other ECM components and thus function to localize latent TGF- $\beta$  in the  
140 ECM. LAP contains an integrin-binding site (RGD), and several RGD-binding integrins are  
141 able to activate latent TGF- $\beta$  through binding this site (Munger and Sheppard 2011). A  
142 common pathway for TGF- $\beta$  activation is through integrins;  $\alpha$ V- $\beta$ 6 on the surface of  
143 epithelial and mesothelial cells induces a conformational change by binding to the RGD motif  
144 present in LAP and activate TGF- $\beta$ , inducing adhesion-mediated cell forces that are translated  
145 into biochemical signals which can lead to liberation/activation of TGF- $\beta$  from its latent  
146 complex (Munger et. Al. 1999, Munger and Sheppard 2011). Secondly,  $\alpha$ V- $\beta$ 6 integrin on the

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2  
3 147 surface of epithelial and mesothelial cells can activate latent TGF- $\beta$  by creating a close  
4  
5 148 connection between the latent TGF- $\beta$  complex and matrix metalloproteinase (MMP)-2 and  
6  
7 149 MMP-9, which can activate TGF- $\beta$  through proteolytic degradation of the LAP (Yu &  
8  
9 150 Stamenkovic 2000; Mu et al. 2002; Annes 2003; Wipff and Hinz 2008). Notably integrin  $\alpha$ V  
10  
11 151 and  $\beta$ 6 null mice both display similar phenotypes to the *Tgfb1* null mice (Bader et al. 1998;  
12  
13 152 Huang et al. 1996; Shull et al. 1992). In addition to MMPs, other proteases, including  
14  
15 153 plasmin, have been shown to activate TGF- $\beta$  ligands through proteolytic degradation (Yu &  
16  
17 154 Stamenkovic 2000; Annes 2003), together with other factors including an acidic pH, which  
18  
19 155 denatures the LAP (Lyons et al. 1988), and thrombospondin-1, which induces a  
20  
21 156 conformational change in LAP thus leading to activation of TGF- $\beta$  ligands (Schultz-Cherry &  
22  
23 157 Murphy-Ullrich 1993). Additional pathways may also lead to the activation of TGF- $\beta$  ligands,  
24  
25 158 and the diverse range of TGF- $\beta$  activation pathways demonstrates that this is a key step in the  
26  
27 159 regulation of TGF- $\beta$  signalling. Annes has published a comprehensive review on TGF- $\beta$   
28  
29 160 activation and regulation, which describes these processes and their importance in more depth  
30  
31 161 (Annes 2003).

32  
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34 162  
35  
36 163 Classically, activated TGF- $\beta$  ligands bind to the constitutively active transmembrane receptor,  
37  
38 164 TGF- $\beta$  receptor II (TGF- $\beta$ RII), which induces a conformational change and initiates the  
39  
40 165 recruitment of transmembrane TGF- $\beta$  receptor I (TGF- $\beta$ RI) (Figure 1) (Shi & Massague  
41  
42 166 2003). The TGF- $\beta$  receptor complex then in turn phosphorylates the receptor regulated  
43  
44 167 transcription factors *SMAD2* and *SMAD3* (Figure 1) (Shi & Massague 2003). A third TGF- $\beta$   
45  
46 168 receptor, TGF- $\beta$  receptor III (TGF- $\beta$ RIII), has been described and was originally thought to  
47  
48 169 be a TGF- $\beta$  co-receptor, presenting TGF- $\beta$  ligands to TGF- $\beta$ RII (Cheifetz et al. 1988). More  
49  
50 170 recently, it has been shown that *Tgfb-RIII* null mice die at gestational day 13.5 indicating  
51  
52 171 TGF- $\beta$ RIII to be an essential component of the TGF- $\beta$  signalling pathway in development  
53  
54 172 (Compton et al. 2007). However, little is known about the role of this receptor in TGF- $\beta$   
55  
56 173 signalling.

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5 175 Phosphorylated receptor Smads form a heteromeric complex of two receptor Smads together  
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7 176 with the co-Smad, Smad4, before nuclear translocation and regulation of transcriptional  
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9 177 responses (Figure 1) (Schmierer & Hill 2007). Smad-mediated transcription can be either  
10  
11 178 positive or negative and is thought to occur through chromatin remodelling and histone  
12  
13 179 modification rather than direct recruitment of transcriptional machinery (Ross et al. 2006; Shi  
14  
15 180 & Massague 2003). Inhibitory Smad7 mediates negative feedback in the TGF- $\beta$  signalling  
16  
17 181 pathway by competing for TGF- $\beta$  receptor I binding and inhibiting phosphorylation of Smad2  
18  
19 182 or Smad3 (Schmierer & Hill 2007). TGF- $\beta$  signalling through Smad independent pathways,  
20  
21 183 such as tyrosine kinase and G-protein-coupled signalling pathways, has been described,  
22  
23 184 although the links between the activated TGF- $\beta$  receptors and the downstream signalling  
24  
25 185 molecules remain unknown in most cases (Moustakas 2005). Additionally, TGF- $\beta$  signalling  
26  
27 186 through the nodal signalling pathway, a crucial embryogenesis pathway, has been described  
28  
29 187 in tumorigenesis (Quail et al. 2013; Schmierer & Hill 2007; (Moustakas 2005).  
30  
31 188 TGF- $\beta$  signalling elicits a wide variety of downstream processes, however this is in direct  
32  
33 189 contrast with the number of Smad proteins recruited by the TGF- $\beta$  receptors and it is not fully  
34  
35 190 understood how TGF- $\beta$  ligands can produce a variety of distinct responses (Shi & Massague  
36  
37 191 2003). Several theories exist that attempt to explain these responses. Firstly; it has been  
38  
39 192 reported that distinct signal intensities can stimulate differential gene expression, for example,  
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41 193 the nuclear concentration of a transcriptional activator required for expression is determined  
42  
43 194 by the binding affinity of a target gene promoter (Schmierer & Hill 2007). Secondly, differing  
44  
45 195 concentrations of TGF- $\beta$  ligands can activate different responses in gene expression  
46  
47 196 (Schmierer & Hill 2007). Thirdly, the establishment of reciprocal gradients of repressor gene  
48  
49 197 expression have been reported for some genes. Schmierer and Hill describe these processes in  
50  
51 198 more detail (Schmierer and Hill 2007). More recently, a cell-type-specific master  
52  
53 199 transcription factor which directs different responses to Smad2 or Smad3 in different cell  
54  
55 200 types has been reported (Mullen et al. 2011). The mechanism that determines phosphorylation  
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3 201 of Smad2 over Smad3, or vice-versa, by the TGF- $\beta$  receptor in a particular cell type is not yet  
4  
5 202 known (Shi & Massague 2003).  
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7 203

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9  
10 204 **TGF- $\beta$  expression in women with peritoneal endometriosis**

11  
12 205 Several studies have reported significantly higher levels of TGF- $\beta$ 1 in serum, peritoneal fluid,  
13  
14 206 peritoneum and eutopic endometrial tissue of women with endometriosis when compared to  
15  
16 207 women without endometriosis, suggesting that altered TGF- $\beta$  expression and/or signalling  
17  
18 208 may contribute to the pathophysiology of endometriosis ( Oosterlynck et al. 1994; Chegini et  
19  
20 209 al. 1994;Kupker et al. 1998; Pizzo et al. 2002; Fan et al. 2005; Young et al. 2014a; Young et  
21  
22 210 al. 2014b).  
23

24  
25 211 Peritoneal mesothelial cells are the largest cell population within the peritoneal cavity and are  
26  
27 212 reported to overexpress TGF- $\beta$ , and in particular TGF- $\beta$ 1 ligands, into the peritoneal fluid in  
28  
29 213 response to peritoneal related pathologies, such as fibrosis and peritoneal cancers, suggesting  
30  
31 214 that they may play a significant role in the elevated levels of TGF- $\beta$ 1 found in women with  
32  
33 215 endometriosis (Offner et al. 1996). Recently, we have described the peritoneal mesothelial  
34  
35 216 cells as a source of TGF- $\beta$ 1 in the pathology of endometriosis through a series of  
36  
37 217 immunohistochemical staining on primary human peritoneal biopsies and through studies *in*  
38  
39 218 *vitro* of primary peritoneal mesothelial cells (Young et al. 2014b). Additional sources of  
40  
41 219 peritoneal fluid TGF- $\beta$  in women with endometriosis are thought to be from shed menstrual  
42  
43 220 tissue, ectopic endometrial cells and macrophages (Omwandho et al. 2010). The peritoneum  
44  
45 221 from women with endometriosis has been reported to express significantly higher levels of  
46  
47 222 TGF- $\beta$ 1, TGF- $\beta$ 3 and Smad3 than the peritoneum from control women with benign ovarian  
48  
49 223 tumours (Li et al. 2011). However the nature of the cells contributing to this increase (either  
50  
51 224 immune cells, nerve cells, endothelial cells or mesothelial cells), is not clear (Li et al. 2011).  
52  
53 225 Furthermore, as the control group of women included in this study presented with benign  
54  
55 226 ovarian tumours, it is not clear if the observed differences in TGF- $\beta$  ligand and Smad3  
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3 227 expression were linked to the presence of endometriosis or the presence of ovarian pathology  
4  
5 228 (Li et al. 2011). We have recently described a significant increase in TGF- $\beta$ 1 mRNA  
6  
7 229 expression in the peritoneum adjacent to endometriosis lesions, when compared to  
8  
9 230 peritoneum from sites distal to lesions in women with endometriosis. We found no change in  
10  
11 231 mRNA expression of TGF- $\beta$  signalling components (TGF- $\beta$  receptors 1, 2 and Smad3) in the  
12  
13 232 same tissue set, suggesting that the local increase in TGF- $\beta$ 1 may have downstream  
14  
15 233 consequences on TGF- $\beta$  signalling targets within the peritoneum (Young et al. 2014b).  
16

17  
18 234  
19  
20 235 TGF- $\beta$ 1, 2 and 3 are expressed in the human endometrium and their expression is cyclically  
21  
22 236 regulated, with all 3 isoforms being expressed during menstruation and found in shed  
23  
24 237 endometrial tissue. Immunohistochemical analysis showed that TGF- $\beta$ 1 was localised within  
25  
26 238 the stromal cells, glandular cells and macrophages of endometrial tissue, and TGF- $\beta$ 2 and 3  
27  
28 239 have been localised to the stromal cells and glandular cells of the endometrium (Chegini et al.  
29  
30 240 1994; Johnson et al. 2005).. Additionally, TGF- $\beta$ 1 protein levels are significantly increased in  
31  
32 241 the nerve fibres of peritoneal endometriosis lesions, when compared to nerve fibres in  
33  
34 242 peritoneum from women without endometriosis, and a statistically significant relationship  
35  
36 243 was found between TGF- $\beta$ 1 expression and dysmenorrhea (Tamburro et al. 2003).  
37

38 244  
39  
40 245 Despite conclusive evidence that TGF-beta isoforms are expressed and play a crucial  
41  
42 246 signalling role in human endometrium, there is no literature directly showing TGF-beta  
43  
44 247 expression and localisation to endometriosis lesion tissue. It is also not yet known if the  
45  
46 248 increased levels of TGF- $\beta$ 1 in the peritoneal fluid of women with endometriosis precedes or  
47  
48 249 follows the development of endometriosis. However, as retrograde menstruation and the  
49  
50 250 presence of endometrial cells within the peritoneal cavity can induce inflammation and TGF-  
51  
52 251  $\beta$  is an inflammatory cytokine, the development of endometriosis and the increase in TGF- $\beta$ 1  
53  
54 252 are likely to go hand-in-hand ( D'Hooghe et al. 2001a; D'Hooghe et al. 2001b; Li et al. 2011).  
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3 254 Only two of the reported studies indicated whether total or bioactive levels of TGF- $\beta$ 1 were  
4  
5 255 measured, with both reporting only total levels to be measurable in peritoneal fluid,  
6  
7 256 suggesting TGF- $\beta$  ligands are activated locally, and therefore it is important to investigate the  
8  
9 257 local changes induced by the presence of endometriosis lesions in the activation of TGF- $\beta$   
10  
11 258 (Oosterlynck et al. 1994; Young et al. 2014a). One study has examined activation of TGF- $\beta$   
12  
13 259 in women with endometriosis, and this was via the plasminogen activation pathway, which  
14  
15 260 the authors found to be increased at sites of endometriosis lesions, suggesting that there may  
16  
17 261 be more TGF- $\beta$  activity in endometriosis lesions and the surrounding peritoneum (Komiya  
18  
19 262 et al. 2007). Several other activation pathways are likely to play a role in peritoneal TGF- $\beta$   
20  
21 263 ligand activation and may be altered in women with endometriosis. Peritoneal mesothelial  
22  
23 264 cells and endometriosis lesions express several integrins, including integrin  $\alpha$ V and  $\beta$ 6 which  
24  
25 265 are known activators of TGF- $\beta$  ligands, as described above (Odor 1954; Bardi & Hope 1964;  
26  
27 266 van der Linden et al. 1994). These factors may contribute to the activation of TGF- $\beta$  ligands  
28  
29 267 within the local peritoneal environment and changes in integrin expression in women with  
30  
31 268 endometriosis may lead to an increase in TGF- $\beta$  activity. However, despite this pathway  
32  
33 269 being a credible mechanism for TGF- $\beta$  ligand activation in women with endometriosis, it has  
34  
35 270 not yet been investigated in the pathophysiology of endometriosis.  
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41 271  
42  
43 272 TGF- $\beta$ 1 levels may be cyclically regulated within the peritoneal fluid of women and levels of  
44  
45 273 TGF- $\beta$ 1 are significantly increased in the peritoneal fluid of women with endometriosis when  
46  
47 274 compared to women without disease (Kupker et al. 1998; Pizzo et al. 2002; Oosterlynck et al.  
48  
49 275 1994; Young et al. 2014a; Young et al. 2014b). Recently, we reported TGF- $\beta$ 2 and TGF- $\beta$ 3  
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51 276 to be present within the peritoneal fluid, however levels of these ligands remained unchanged  
52  
53 277 between women with and without endometriosis (Young et al. 2014b).  
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58 279 Interestingly, only two studies have quantified serum levels of TGF- $\beta$  in women with  
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60 280 endometriosis compared to women without. Pizzo et al. (2002) examined the levels of TGF- $\beta$

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3 281 using ELISA in serum and peritoneal fluid isolated from 26 women with endometriosis and  
4  
5 282 described a significant increase in serum-TGF- $\beta$  concentrations, which increased with the  
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7 283 severity of the disease and in a similar fashion to peritoneal fluid levels of TGF- $\beta$ . However,  
8  
9 284 this study made no distinction between TGF- $\beta$  isoforms measured and it is not clear if this is  
10  
11 285 TGF- $\beta$ 1 or all TGF- $\beta$  ligands (Pizzo et al. 2002). Another study from a different group where  
12  
13 286 authors have investigated the association between endometriosis and TGF- $\beta$ 1 gene  
14  
15 287 polymorphisms using restriction fragment length polymorphism analysis and serum TGF- $\beta$ 1  
16  
17 288 levels in Korean women, independently confirmed that serum TGF- $\beta$ 1 levels were  
18  
19 289 significantly higher in Korean women with endometriosis (n=120) than in controls (n= 89)  
20  
21 290 (Lee et al. 2011). Both studies described a significant increase in TGF- $\beta$  or TGF- $\beta$ 1 in the  
22  
23 291 serum of women with endometriosis compared to controls, suggesting TGF- $\beta$  may be a  
24  
25 292 potential biomarker for the detection of endometriosis.  
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30  
31 294 Endometriotic lesions express TGF- $\beta$ 1, 2 and 3, in differing protein concentrations, with  
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33 295 TGF- $\beta$ 1 being the most abundantly expressed TGF $\beta$  protein isoform (Chegini et al. 1994).  
34  
35 296 TGF- $\beta$ 1 was shown to be expressed in all cell types, except endometrial stromal cells, found  
36  
37 297 within surgically induced endometriosis lesions in a rat (Chegini et al. 1994). One study  
38  
39 298 demonstrated TGF- $\beta$  mRNA expression to be increased in endometriosis lesion tissue when  
40  
41 299 compared to eutopic endometrial tissue, however it is not clear if the endometrial control  
42  
43 300 tissue is from women with or without endometriosis and the TGF- $\beta$  isoforms measured are  
44  
45 301 not reported (Fan et al. 2005). The TGF- $\beta$  signal transducers Smad3, pSmad3, and Smad4,  
46  
47 302 and the inhibitory Smad7 proteins were also observed in the endometrial stromal and  
48  
49 303 epithelial cells (Luo et al., 2003a) and suggest a role for TGF- $\beta$ s in the normal function of the  
50  
51 304 human endometrium. In eutopic endometrium transcriptional activity of Smad3 is suppressed  
52  
53 305 by the estrogen receptor (ER) in an estradiol-dependent manner, and ER-mediated  
54  
55 306 transcription increases after activation of TGF- $\beta$  signaling (Matsuda *et al.*, 2001; Cherlet and  
56  
57 307 Murphy, 2007). Studies have also shown that eutopic endometrium express Smads and that  
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3 308 TGF- $\beta$ 1 increases both the expression of Smad3, and the phosphorylation of Smad3 *in vitro*  
4  
5 309 in a dose-dependent manner, suggesting endometriotic cells may also be responsive to TGF-  
6  
7 310  $\beta$ 1 signalling (Luo et al. 2003b). TGF- $\beta$ 1 was shown to be aberrantly expressed in the  
8  
9 311 endometrium of women with endometriosis when compared to women without endometriosis,  
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11 312 an observation the authors suggested may be linked to the increased cell proliferation seen in  
12  
13 313 the endometrial cells of women with endometriosis (Johnson et al. 2005).  
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17 315 Recently, Hull et al. described a reduced growth of endometriosis lesions in TGF- $\beta$ 1-null  
18  
19 316 mice when compared to their wild-type counterparts, demonstrating TGF- $\beta$ 1 to play a key  
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21 317 role in endometriosis lesion development (Hull et al. 2012) and tissue repair and remodelling  
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23 318 (Hull et al. 2008). These studies have been summarised in Table 1.  
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### 27 28 29 320 **A role for TGF- $\beta$ 1 in the pathophysiology of peritoneal endometriosis**

30  
31 321 Although TGF- $\beta$ 1 expression appears to be increased in women with endometriosis compared  
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33 322 to women without endometriosis, less is known about the functional role of TGF- $\beta$ 1 in the  
34  
35 323 development and maintenance of peritoneal endometriosis. TGF- $\beta$ 1 is a multifunctional  
36  
37 324 cytokine, which is known to regulate a variety of biological processes e.g. cell proliferation,  
38  
39 325 ECM formation, tissue remodeling, and inflammation (Massague et al. 2000; Jakowlew  
40  
41 326 2006). Similar biological events occur during endometriotic lesion establishment, and  
42  
43 327 although there is little understanding of the signaling events that control them, there is  
44  
45 328 evidence of TGF- $\beta$ 1 involvement. Herein follows a review of the possible functional roles for  
46  
47 329 TGF- $\beta$ 1 in the pathophysiology of peritoneal endometriosis. These functions are summarised  
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49 330 in Table 2.

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52 331 TGF- $\beta$  regulation of ectopic endometrial cell survival

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54 332 TGF- $\beta$ 1, together with its downstream signalling targets involved in cell survival, including  
55  
56 333 mRNA expression levels of *BAX* and *C-MYC*, were shown to be altered in the eutopic  
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3 334 endometrium of women with endometriosis compared to women without disease (Johnson et  
4  
5 335 al. 2005), suggesting that increased TGF- $\beta$ 1 may lead to increased apoptosis resistance in the  
6  
7 336 shed endometrial tissue of women with endometriosis. The change in *BAX* and *C-MYC*  
8  
9 337 expression may facilitate survival of ectopic endometrial tissue during transport to the  
10  
11 338 peritoneal cavity (Johnson et al. 2005). Furthermore, the increasing concentrations of TGF- $\beta$ 1  
12  
13 339 in the peritoneal fluid of women with endometriosis may further contribute to the expression  
14  
15 340 of anti-apoptotic factors in shed endometrial tissue (Seoane 2006). *Tgfb1* null mice showed  
16  
17 341 reduced numbers of endometrial epithelial cells, without any observed changes in cell  
18  
19 342 proliferation, leading to the hypothesis that TGF- $\beta$ 1 may be responsible for inducing anti-  
20  
21 343 apoptosis effects within these cells, supporting this theory (Hull et al. 2012).

22  
23 344 Together with increased apoptosis resistance and decreases in immune cell numbers and  
24  
25 345 activity within the peritoneal fluid and peritoneum, TGF- $\beta$  may also contribute to ectopic  
26  
27 346 tissue survival. TGF- $\beta$ 1 overexpression has been linked to a reduction in peritoneal fluid and  
28  
29 347 peritoneal tissue natural killer (NK) cell and macrophage numbers leading to suppressed  
30  
31 348 scavenger function in the peritoneum (Hull et al. 2012; Mizumoto 1996; Dou et al. 1997).  
32  
33 349 This could limit the clearance of retrograde menstrual tissue within the peritoneal cavity and  
34  
35 350 may lead to a greater chance of ectopic endometrial cell survival.

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39 351 TGF- $\beta$  regulation of ectopic endometrial cell attachment onto the peritoneum

40  
41 352 Adhesion of human endometrial cells to mouse peritoneum is increased on exposure to TGF-  
42  
43 353  $\beta$ 1 in an *in-vitro* co-culture attachment assay (Beliard et al. 2003), but the mechanism by  
44  
45 354 which TGF- $\beta$ 1 treatment increases ectopic cell adhesion is unclear. It may be induced by  
46  
47 355 altered expression of cell surface adhesion molecules on the peritoneal mesothelial cells,  
48  
49 356 changes in the morphology of the peritoneal mesothelial cells exposing the underlying  
50  
51 357 peritoneal tissue or altered expression of cell surface adhesion molecules on the endometrial  
52  
53 358 cells, or a combination of some or all of the above (Beliard et al. 2003). As discussed  
54  
55 359 previously, the mechanism of ectopic endometrial cell attachment to the peritoneum and the  
56  
57 360 site of ectopic endometrial cell attachment, either directly to the peritoneal mesothelium or to  
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3 361 the underlying connective tissue, is not fully understood (Dunselman et al. 2001). Another  
4  
5 362 study using a functional co-culture adhesion assay showed conflicting results, with exposure  
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7 363 to 5ng TGF- $\beta$ 1 significantly increasing attachment of EM42 endometrial epithelial cells to  
8  
9 364 LP9 peritoneal mesothelial cells, but this was not reproducible for the primary endometrial  
10  
11 365 epithelial cells (Liu et al. 2009). Additionally, exposure to 10ng TGF- $\beta$ 1 significantly reduced  
12  
13 366 the attachment of primary endometrial epithelial cells to LP9 peritoneal mesothelial cells, but  
14  
15 367 this was not reproducible for the EM42 cell line (Liu et al. 2009); in this study, the authors  
16  
17 368 pre-treated the endometrial epithelial cells with TGF- $\beta$ 1. An interesting follow-up study  
18  
19 369 would be to repeat the attachment assay with pre-treated peritoneal mesothelial cells, as these  
20  
21 370 cells will also be in direct contact with the peritoneal fluid and hence the increased TGF- $\beta$ 1  
22  
23 371 concentrations in women with endometriosis. Moreover the peritoneal mesothelium is a key  
24  
25 372 defensive barrier: therefore it is more likely that changes within the peritoneal mesothelial  
26  
27 373 cells than changes to the ectopic endometrial cells increase ectopic cell adhesion and  
28  
29 374 invasion.

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33 375 TGF- $\beta$  regulation of ectopic endometrial cell invasion into the peritoneum

34  
35 376 Studies have shown that TGF- $\beta$ 1 enhances ectopic endometrial cell invasion into peritoneal  
36  
37 377 tissue during the development of endometriosis lesions (Liu et al. 2009). Using a three-  
38  
39 378 dimensional cell invasion assay model, Liu et al. demonstrated that TGF- $\beta$ 1 dose-dependently  
40  
41 379 increases invasion of EM42 endometrial epithelial cells and primary endometrial epithelial  
42  
43 380 cells through a monolayer of LP9 peritoneal mesothelial cells, and this effect is inhibited by  
44  
45 381 addition of a TGF- $\beta$ R1 antagonist (Liu et al. 2009). The endometrial epithelial cells were pre-  
46  
47 382 treated with either TGF- $\beta$ 1 and/or TGF- $\beta$ R1 antagonist, showing the effects of TGF- $\beta$ 1 on  
48  
49 383 ectopic endometrial cells. This study demonstrates that TGF- $\beta$ 1 is able to increase  
50  
51 384 endometrial epithelial cell invasion and results suggest that TGF- $\beta$ 1 maybe inducing  
52  
53 385 epithelial to mesenchymal transition (EMT) within these cells, which would explain the  
54  
55 386 increased migratory and invasion capacity (Liu et al. 2009). EMT within the peritoneal  
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3 387 mesothelial cells has also been discussed in the pathophysiology of endometriosis by  
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5 388 increasing ectopic endometrial cell attachment or invasion into the peritoneum through  
6  
7 389 disruption of the mesothelial monolayer (Dunselman et al. 2001; Weusten et al. 2000; Demir  
8  
9 390 et al. 2004).

10  
11 391 TGF- $\beta$ 1 is the most well-known inducer of EMT and one group has demonstrated that TGF-  
12  
13 392  $\beta$ 1 may be a cause of EMT within the ectopic endometrial epithelial cells of endometriosis  
14  
15 393 lesions in baboons and this was linked to increased cellular contractility and lesion-associated  
16  
17 394 fibrosis (Zhang et al. 2016a) In a follow-up baboon study, TGF- $\beta$ 1 was confirmed to induce  
18  
19 395 EMT within ectopic endometrial epithelial cells and immunohistochemical analysis has  
20  
21 396 shown that concentrations of TGF- $\beta$ 1 and pSmad3 were correlated with the extent of fibrosis  
22  
23 397 (Zhang et al. 2016b).

24  
25  
26  
27 398 TGF- $\beta$ 1 regulation of peritoneal immune cell activity

28  
29 399 TGF- $\beta$ 1 autocrine and paracrine signalling within peritoneal macrophage populations were  
30  
31 400 shown to play an essential role in the development of endometriosis lesions (Dou et al. 1997).

32  
33 401 In-vitro functional assays showed that TGF- $\beta$ 1 regulates macrophage DNA synthesis and cell  
34  
35 402 proliferation, macrophage cell-cell interaction and mRNA expression of several macrophage  
36  
37 403 cell surface adhesion molecules, including: integrins  $\alpha$ 2,  $\alpha$ 3,  $\alpha$ 4,  $\alpha$ v,  $\beta$ 1,  $\beta$ 6 and platelet-  
38  
39 404 endothelial cell adhesion molecule-1 (Dou et al. 1997). Blocking TGF- $\beta$  expression and  
40  
41 405 signalling in these cells using TGF- $\beta$ 1 antisense oligomers prevented these effects (Dou et al.  
42  
43 406 1997). TGF- $\beta$  expression by peritoneal macrophages may also regulate integrin expression  
44  
45 407 both within the ectopic endometrial cells and the peritoneal mesothelial cells, contributing to  
46  
47 408 ectopic endometrial cell attachment to the peritoneum, however this mechanism has not yet  
48  
49 409 been discussed within endometriosis literature. Interestingly, peritoneal endometriosis lesions  
50  
51 410 from Tgfb1-null mice contained significantly reduced numbers of macrophages, when  
52  
53 411 compared to wild-type control mice, suggesting that TGF- $\beta$ 1 is responsible for the  
54  
55 412 recruitment of peritoneal macrophages into endometriosis lesions (Hull et al. 2012). TGF- $\beta$   
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3 413 signalling has been shown to promote M2-type macrophages activation, which are involved  
4  
5 414 in inflammation, tissue repair and promote removal of apoptotic cells (Depeng Gong 2012).  
6  
7 415 Recently it was shown that M1-type macrophages suppress endometriotic lesion  
8  
9 416 development, whereas M2-type macrophages, associated with wound healing and tissue  
10  
11 417 remodeling, enhance lesion development (Bacci et al. 2009).  
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15 419 Decreased NK cell activity within the peritoneal cavity in women with endometriosis has  
16  
17 420 been attributed to increasing concentrations of TGF- $\beta$  within the peritoneal fluid (Mizumoto  
18  
19 421 1996). Furthermore, peritoneal fluid from women with endometriosis, or treatment with TGF-  
20  
21 422  $\beta$ , inhibited the development of mice embryos and this was attributed to the decrease in NK  
22  
23 423 cell activity, although again the TGF- $\beta$  isoforms measured or used in treatments is unknown  
24  
25 424 (Mizumoto 1996). Nevertheless, these results do suggest that increasing TGF- $\beta$  levels in the  
26  
27 425 peritoneal fluid, and potentially the endometrium of women with endometriosis, may have an  
28  
29 426 adverse effect on fertility.  
30

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34  
35 428 TGF- $\beta$ 1 regulation of ectopic endometrial cell proliferation

36  
37 429 Traditionally, TGF- $\beta$ 1 has been known to have anti-proliferative effects on epithelial cells but  
38  
39 430 proliferative effects on stromal cells (Seoane 2006). In a mouse model of endometriosis, Hull  
40  
41 431 et al. (2012) demonstrated no change in endometrial epithelial or stromal cell proliferation in  
42  
43 432 Tgf- $\beta$ 1 deficient mice, compared to wild-type mice, using BrdU staining. The mouse model  
44  
45 433 utilised within this study used endometrial tissue from human subjects and therefore the  
46  
47 434 endometrial cells themselves were not Tgf- $\beta$ 1 deficient, which may explain why no change in  
48  
49 435 cell proliferation was observed. Supporting the finding that TGF- $\beta$ 1 does not regulate  
50  
51 436 endometriotic cell proliferation, an in-vitro assay demonstrated TGF- $\beta$ 1 exposure has no  
52  
53 437 effect on endometrial epithelial cell proliferation, either in primary endometrial epithelial  
54  
55 438 cells or in the EM42 endometrial epithelial cell line, across several concentrations (Beliard et  
56  
57 439 al. 2003). Conversely, in another study TGF- $\beta$ 1 was shown to increase protease activated  
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3 440 receptor 2 (*PAR2*) mRNA expression and activation in endometrial stromal cells (Saito et al.  
4  
5 441 2011), and *PAR2* has been shown to induce proliferation of endometrial stromal cells  
6  
7 442 (Yasushi Hirota 2005). Par2-deficient mice also develop smaller and fewer endometriosis  
8  
9 443 lesions than wild-type counterparts, suggesting a role for TGF- $\beta$ 1-mediated Par2 expression  
10  
11 444 in ectopic endometrial cell proliferation (Osuga et al. 2008).

#### 14 445 TGF- $\beta$ 1 regulation of neoangiogenesis

16 446 Neoangiogenesis is a critical step in the pathophysiology of endometriosis and studies have  
17  
18 447 demonstrated that blocking angiogenesis can block the establishment or growth of  
19  
20 448 endometriosis lesions in a murine model of endometriosis (Laschke 2005; Hull et al. 2003).  
21  
22 449 At a macroscopic level, lesions have been shown to be highly vascularised with new vessels  
23  
24 450 developing from the surrounding peritoneum in hamsters (Overton et al. 2007). Vascular  
25  
26 451 endothelial growth factor-A (VEGF-A) is the most potent angiogenic factor, which is  
27  
28 452 increased in the peritoneal fluid of women with endometriosis compared to women without  
29  
30 453 the disease (McLaren et al. 1996; Kupker et al. 1998; Young et al. 2015). TGF- $\beta$ 1 is an  
31  
32 454 established regulator of VEGF expression in several cell types and overexpression of TGF- $\beta$   
33  
34 455 and VEGF has been implicated in neoangiogenesis of several cancers (Kaminska et al. 2005).  
35  
36 456 We have shown that the protein concentrations of VEGF-A in the peritoneal fluid of women  
37  
38 457 with and without endometriosis correlate with concentrations of TGF- $\beta$ 1, suggesting a  
39  
40 458 regulatory role for TGF- $\beta$ 1 in the peritoneal expression of VEGF-A (Young et al. 2015). In  
41  
42 459 the same study, we demonstrated that TGF- $\beta$ 1 may be responsible for the increase in  
43  
44 460 secretion of VEGF-A from the peritoneal mesothelium through the Inhibitor of DNA Binding  
45  
46 461 Protein 1 (*IDI*) pathway, in a similar mechanism to several epithelial cancers, thus  
47  
48 462 contributing to the vascularisation of endometriosis lesions (Young et al. 2015).

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3 464 **TGF- $\beta$ 1 regulation of ectopic endometrial and peritoneal mesothelial cell**

4  
5 465 **metabolism**

6  
7 466 Ectopic endometrial tissue must survive in a hypoxic environment during peritoneal transport,  
8  
9 467 attachment and invasion into the peritoneum, much like metastatic cancer cells. During  
10  
11 468 tumour development and metastasis, glycolysis is initially used for energy production, owing  
12  
13 469 to the hypoxic conditions. Although tumours will eventually develop a blood supply and  
14  
15 470 hence a supply of oxygen, tumour cells continue to use glycolysis as their main source of  
16  
17 471 energy production and this phenotype is often referred to as the 'Warburg effect' (Gatenby &  
18  
19 472 Gillies 2004). Side effects of glycolysis include an increase in cell proliferation and motility,  
20  
21 473 breakdown of ECM and a resistance to apoptosis all of which contribute towards the  
22  
23 474 progression of the disease (Gatenby & Gillies 2004). The Warburg effect is induced by  
24  
25 475 inflammatory cytokines, including TGF- $\beta$ 1, via the induction of hypoxia inducible factor  
26  
27 476 (HIF)-1 $\alpha$  protein expression under normoxic conditions (Fosslien 2008; Guido et al. 2012).

28  
29 477 There are observations in the literature that suggest ectopic endometrial tissue is using  
30  
31 478 glycolysis as a means of energy production, such as absence of glycogen deposits, the  
32  
33 479 presence of small mitochondria and resistance to apoptosis (Jones et al. 2009). Furthermore,  
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35 480 studies have shown *HIF-1 $\alpha$*  to be expressed in endometriosis lesions and HIF-1 $\alpha$  mRNA and  
36  
37 481 protein expression levels are significantly increased in lesions when compared to matched  
38  
39 482 eutopic endometrium and healthy control endometrium (Ren et al. 2007; Wu et al. 2007),  
40  
41 483 although these studies did not link the reported findings to changes in endometriotic cell  
42  
43 484 metabolism.

44  
45 485 We described for the first time potential changes in the cellular metabolism of ectopic  
46  
47 486 endometrial tissue and the surrounding peritoneal tissue of endometriosis lesions, similar to  
48  
49 487 that of the Warburg effect seen in tumourigenesis (Young et al. 2014a). In this study we  
50  
51 488 described significantly higher levels of lactate within the peritoneal fluid of women with  
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53 489 endometriosis and we reported a significant positive correlation between concentrations of  
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55 490 lactate and TGF- $\beta$ 1. These findings were backed up with work *in vitro* demonstrating that  
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3 491 TGF- $\beta$ 1 increases lactate concentrations in primary peritoneal mesothelial cells and a  
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5 492 mesothelial cell line, suggesting that TGF- $\beta$ 1 may regulate changes in cell metabolism that  
6  
7 493 may fuel ectopic endometrial cell survival and endometriosis lesion development (Young et al.  
8  
9 494 2014a). In a follow up study we demonstrated that TGF- $\beta$ 1 induces changes in the metabolic  
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11 495 phenotype through the inhibitor of DNA-binding protein 2 (*ID2*) pathway (Young et al.  
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13 496 2016).  
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### 18 498 **The clinical significance for TGF- $\beta$ 1 in peritoneal endometriosis**

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21 499 Therapeutic moderators of TGF- $\beta$  expression in endometriosis  
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24 500 GnRH analogues (GnRHa) are commonly used in the medical management of endometriosis  
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26 501 (Panay 2008). While the primary effect is in blocking the production of sex steroids from the  
27  
28 502 ovary, endometrial stromal cells express GnRH receptors and GnRHa can act directly on  
29  
30 503 these cells, inducing changes in gene expression, including the expression of TGF- $\beta$  isoforms  
31  
32 504 and their receptors (Chegini et al. 2003). Therefore, treatment with GnRHa may have  
33  
34 505 additional efficacy in the treatment of endometriosis by decreasing the expression and  
35  
36 506 signalling of TGF- $\beta$ . TGF- $\beta$  concentrations in the peritoneal fluid from women with  
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38 507 endometriosis was significantly reduced after 4 months of treatment with a GnRHa, although  
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40 508 the *TGFB* isoforms measured were not reported (Kupker et al. 1998). It is not clear how much  
41  
42 509 of this effect is directly mediated by GnRH or indirectly through estrogen removal.  
43  
44 510 Functional studies have looked at the effects of GnRHa and TGF- $\beta$ 1 exposure on the  
45  
46 511 expression of fibronectin by endometrial epithelial cells and stromal cells *in vitro*. Microarray  
47  
48 512 results demonstrated that *TGFB1* significantly increased fibronectin expression, while GnRHa  
49  
50 513 significantly decreased fibronectin gene expression (Chegini et al. 2003). The authors  
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52 514 concluded that as fibronectin is an essential component in the attachment of ectopic  
53  
54 515 endometrium to the peritoneum, this might be a mechanism by which GnRHa therapies  
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56 516 influence the development of endometriosis lesions (Chegini et al. 2003). In a follow-up  
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3 517 study, Luo et al. demonstrated that GnRHa could inhibit phosphorylation of Smad3 in  
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5 518 endometrial stromal cells, suggesting that GnRHa therapies may block *TGF- $\beta$*  signalling in  
6  
7 519 endometrial cells and potentially other cells expressing the GnRH receptors, however whether  
8  
9 520 this is a direct effect of blocking Smad3 phosphorylation by the TGF- $\beta$ RI or indirect by  
10  
11 521 inhibition of TGF- $\beta$  ligand and receptor expression is unclear (Luo et al. 2003a).

### 14 522 **TGF- $\beta$ polymorphisms in women with endometriosis**

16  
17 523 Although the exact aetiology of endometriosis remains unclear, genetic predisposition is  
18  
19 524 thought to play a role. Twin studies have pointed to a genetic component (Montgomery et al.  
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21 525 2008) and women who have a first-degree relative with endometriosis have an increased  
22  
23 526 chance of developing endometriosis themselves (Giudice & Kao 2004). Several studies have  
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25 527 investigated polymorphisms in the *TGFBI* in women with endometriosis, to try and explain  
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27 528 the genetic component of this complex disease. The *TGFBI*-509C/T polymorphism is the  
28  
29 529 most commonly researched polymorphism of the *TGFBI* gene in the context of  
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31 530 endometriosis, as this polymorphism is the main determinant of plasma TGF- $\beta$ 1  
32  
33 531 concentrations (Grainger et al. 1999). The results of these studies are inconsistent, with  
34  
35 532 several studies demonstrating *TGFBI* polymorphisms to be associated with endometriosis,  
36  
37 533 where other studies found no association (Lee et al. 2011; Kim et al. 2010; Hsieh et al. 2005).  
38  
39 534 A recent meta-analysis of the association of the *TGFBI*-509C/T polymorphism and the  
40  
41 535 occurrence of endometriosis found no significant relationship (Zhang et al. 2012). Other  
42  
43 536 polymorphisms, such as the *TGFBI*-868T/C, which has been associated with early-stage  
44  
45 537 endometriosis in Korean women (Lee et al. 2011), may be of future interest in endometriosis  
46  
47 538 research with regards to its functional impact on endometriosis lesion development and as a  
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49 539 candidate gene marker for endometriosis susceptibility.

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53 541 Several genome-wide association studies have now been performed to further investigate the  
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55 542 genetic predisposition associated with endometriosis. A meta-analysis of these studies has  
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57 543 identified eight gene loci to be of possible significance in the pathophysiology of



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2  
3 544 endometriosis (Rahmioglu et al. 2014). However, none of these gene loci belonged to the  
4  
5 545 TGF- $\beta$  superfamily, indicating that there is unlikely to be a direct genetic linkage resulting  
6  
7 546 from the TGF- $\beta$  superfamily (Rahmioglu et al. 2014).  
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11  
12 548 **SUMMARY**  
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15  
16 550 TGF- $\beta$  regulates a variety of cellular functions including cell proliferation, cell adhesion, cell  
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18 551 migration, cell differentiation, apoptosis, angiogenesis and immune cell function. TGF- $\beta$  is  
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20 552 overexpressed in the peritoneal fluid of women with endometriosis compared to women  
21  
22 553 without disease and expression may also be increased in serum, peritoneum, and eutopic  
23  
24 554 endometrium. Although the expression pattern of TGF- $\beta$  is documented in the endometriosis  
25  
26 555 literature, less is reported regarding the functional role(s) that TGF- $\beta$  plays in the  
27  
28 556 development and maintenance of endometriosis lesions. However, new mechanistic studies  
29  
30 557 have recently implicated overexpression of TGF- $\beta$  in several stages of endometriosis lesion  
31  
32 558 development.  
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37 560 Studies have shown that increased levels of TGF- $\beta$ 1 may be responsible for the impaired  
38  
39 561 immune surveillance within the peritoneum of women with endometriosis owing to its ability  
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41 562 to decrease NK cell activity. This decrease in immune surveillance may facilitate ectopic  
42  
43 563 endometrial cell survival within the peritoneal cavity. Furthermore, aberrant TGF- $\beta$ 1  
44  
45 564 expression within eutopic endometrium and peritoneal fluid of women with endometriosis  
46  
47 565 may increase apoptosis resistance in endometrial cells, further fuelling ectopic endometrial  
48  
49 566 cell survival. Attachment of ectopic endometrial cells to the surface of peritoneum and  
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51 567 invasion of ectopic cells through the peritoneal mesothelium may increase on exposure to  
52  
53 568 TGF- $\beta$ 1, although the mechanisms governing this and the cell types altered by TGF- $\beta$   
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55 569 signalling, either peritoneal mesothelial cell or ectopic endometrial cell or both, are not  
56  
57 570 entirely clear. TGF- $\beta$ 1 overexpression may also contribute to ectopic cell survival, invasion  
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3 571 and angiogenesis through changes in cell metabolism to mimic that of cancer cell metabolism,  
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5 572 and finally TGF- $\beta$ 1 may also regulate neoangiogenesis through expression of VEGF-A.

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9 574 Mouse studies using the TGF- $\beta$ 1-null phenotype have given particular insights into the  
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11 575 processes that TGF- $\beta$ 1 is likely to regulate during endometriosis lesion formation. Reduced  
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13 576 numbers of macrophages and myofibroblasts in endometriosis lesions from *Tgfb1* null mice  
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15 577 suggest TGF- $\beta$ 1 regulation of immune and inflammatory responses. However, there were no  
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17 578 observed changes in cell proliferation or blood vessel density, suggesting that TGF- $\beta$ 1 may  
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19 579 not be essential for cell growth or angiogenesis within peritoneal endometriosis lesions,  
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21 580 contradicting the above observations. However, the overall reduced size and number of  
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23 581 endometriosis lesions in *Tgfb1*-null mice compared to wild type mice does indicate that  
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25 582 targeting the TGF- $\beta$  pathway may be of potential therapeutic interest.

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30 584 The overexpression of TGF- $\beta$ 1 in the endometriosis microenvironment may contribute to the  
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32 585 pathophysiology in a similar fashion to its oncogenic effects during tumorigenesis, by  
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34 586 inducing changes in cellular metabolism, increasing cell invasion and initiating  
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36 587 neoangiogenesis. Indeed, the same processes that induce TGF- $\beta$ 's tumour promoting activity  
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38 588 may also be critical in endometriosis lesion development and a switch in TGF- $\beta$  signalling,  
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40 589 from tumour suppressor to tumour promoter, may help explain why some women develop  
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42 590 endometriosis and others do not. Endometriosis is associated with an increased risk of several  
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44 591 cancers, including ovarian cancer, breast cancer and non-Hodgkin's lymphoma, therefore it is  
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46 592 likely that the same causalities or environmental factors which predispose to the development  
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48 593 of endometriosis lesions contribute to the onset of these cancers and vice versa (Kokcu 2011).  
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50 594 This review highlights a key role for TGF- $\beta$ 1 in the pathophysiology of peritoneal  
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52 595 endometriosis and suggests that therapeutic agents which target TGF- $\beta$ 1 expression or its  
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54 596 downstream signalling targets may be beneficial in the prevention and/or treatment of  
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56 597 peritoneal endometriosis.  
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8  
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10

11 601 **Authors' roles**

12  
13 602 All authors (V.J.Y., S.F.A., W.C.D., A.W.H.) contributed equally to the interpretation of the  
14  
15 603 data in the manuscript and the drafting of the manuscript, and have approved the final  
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17 604 version to be published.  
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30 609 **Conflict of Interest**

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32 610 The authors declare they have no conflicts of interest.  
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3 852 **Figure legends**  
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7 854 **Figure 1. The TGF- $\beta$ -Smad signalling pathway.**  
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9 855 Transforming growth factor  $\beta$  (TGF- $\beta$ ) ligands bind to the receptor TGF- $\beta$ RII, resulting in a  
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11 856 conformational change that results in the recruitment of TGF- $\beta$ RI. The TGF- $\beta$  receptor  
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13 857 complex phosphorylates intracellular receptor regulated Smad2 and Smad3, which form a  
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15 858 dimer before coupling with Smad4 and trans-locating to the nucleus where these transcription  
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17 859 factors regulate gene expression. TGF- $\beta$  ligands may also bind the TGF- $\beta$ RIII, which can  
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19 860 present these ligands to the TGF- $\beta$ RII. TGF- $\beta$  signalling through the Smad pathway is known  
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21 861 to have impacts on cell growth, angiogenesis, cell differentiation, apoptosis, invasion,  
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23 862 immune cell recruitment and metabolism. Figure is adapted from Schmierer and Hill (2007).  
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28 864 **Figure 2. Schematic representation of the potential roles of TGF- $\beta$  in the recognised**  
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30 865 **steps leading to the establishment and progression of peritoneal endometriosis.**  
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32 866 HIF-1 $\alpha$  = hypoxia inducible factor  $\alpha$ , BAX = BCL2-associated X protein, EMT = epithelial to  
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34 867 mesenchymal transition, ID1 = inhibitor of DNA binding 1, ID2 = inhibitor of DNA binding  
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36 868 2, VEGF = vascular endothelial growth factor, NK=natural killer.  
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For Peer Review

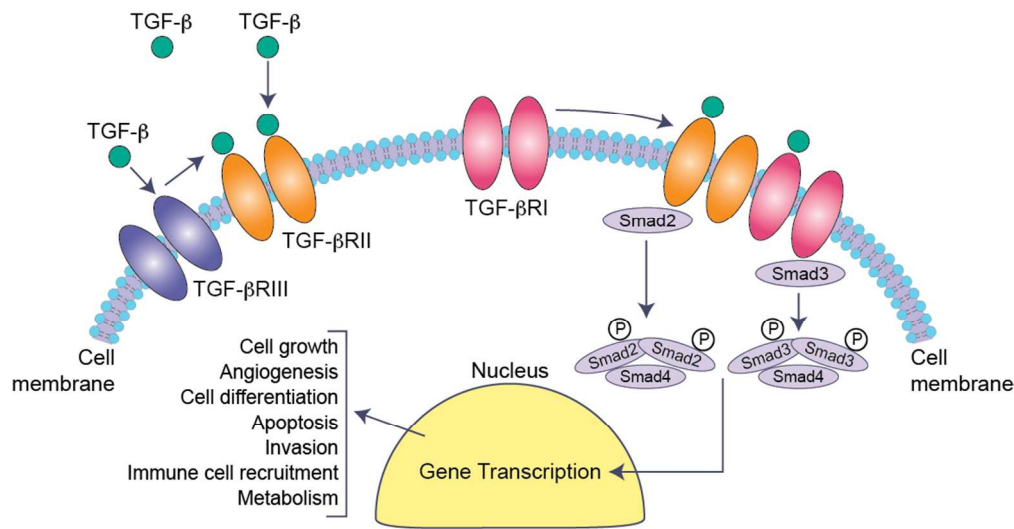
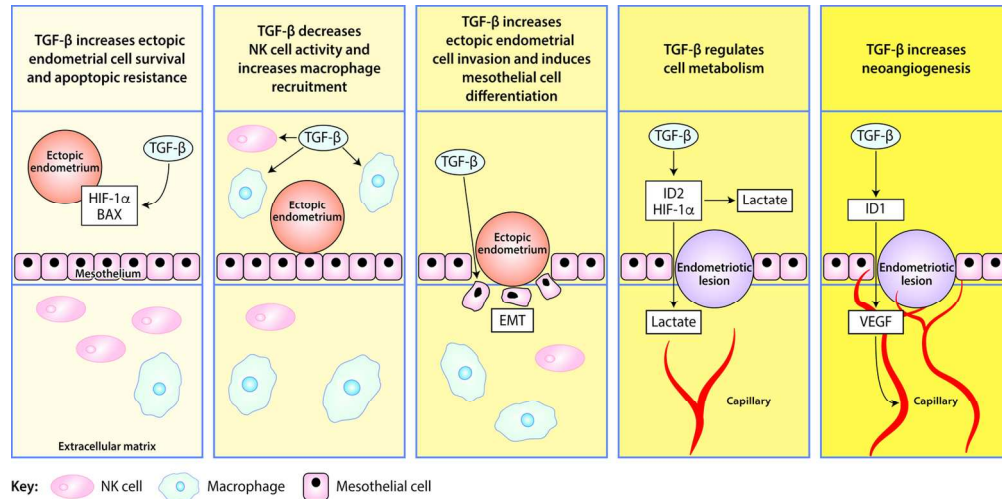


Figure 1 TGF- $\beta$ -Smad signalling pathway. TGF- $\beta$  ligands bind to TGF- $\beta$ RII, resulting in a conformational change that results in the recruitment of TGF- $\beta$ RI. The TGF- $\beta$  receptor complex phosphorylates intracellular receptor regulated Smad2 and Smad3, which form a dimer before coupling with Smad4 and trans-locating to the nucleus where these transcription factors regulate gene expression. TGF- $\beta$  ligands may also bind the TGF- $\beta$ RIII, which can present these ligands to the TGF- $\beta$ RII. TGF- $\beta$  signalling through the Smad pathway is known to have impacts on cell growth, angiogenesis, cell differentiation, apoptosis, invasion, immune cell recruitment and metabolism. Figure is adapted from Schmierer and Hill 2007.

196x101mm (150 x 150 DPI)

Review



Schematic representation of the potential roles of transforming growth factor  $\beta$  in the recognised steps leading to the establishment and progression of peritoneal endometriosis. TGF- $\beta$  = transforming growth factor  $\beta$ ; HIF-1 $\alpha$  = hypoxia inducible factor  $\alpha$ , BAX = BCL2-associated X protein, EMT = epithelial to mesenchymal transition, ID1 = inhibitor of DNA binding 1, ID2 = inhibitor of DNA binding 2, VEGF = vascular endothelial growth factor.

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Review

Ligand	Protein / mRNA	Experimental tissue	Control clinical group	Changes in ligand concentrations in endometriosis	Reference
TGF- $\beta$ 1	Protein	Peritoneal fluid	Pelvic pain	Significantly increased p<0.05	Young et al., 2014a
TGF- $\beta$	Protein	Peritoneal fluid	No pathology	Significantly increased p<0.005	Kupker et al., 1998
TGF- $\beta$	Protein	Peritoneal fluid	Infertility	Significantly increased p<0.001	Pizzo et al., 2002
TGF- $\beta$ 1	Protein	Peritoneal fluid	No pathology	Significantly increased p<0.05	Oosterlynck et al., 1994
TGF- $\beta$ 1	Protein	Peritoneal fluid	Pelvic pain	Significantly increased p<0.05	Young et al., 2014b
TGF- $\beta$ 2	Protein	Peritoneal fluid	Pelvic pain	No significant change	Young et al., 2014b
TGF- $\beta$ 3	Protein	Peritoneal fluid	Pelvic pain	No significant change	Young et al., 2014b
TGF- $\beta$ 1	mRNA	Peritoneum	Pelvic pain	Significantly increased p<0.05	Young et al., 2014b
TGF- $\beta$ 2	mRNA	Peritoneum	Pelvic pain	No significant change	Young et al., 2014b
TGF- $\beta$ 3	mRNA	Peritoneum	Pelvic pain	No significant change	Young et al., 2014b
TGF- $\beta$	Protein	Serum	Infertility	Significantly increased p<0.001	Pizzo et al., 2002
TGF- $\beta$ 1	Protein	Serum	Infertility	Significantly increased p<0.0001	Lee et al., 2011

**Table1.** Studies that have measured transforming growth factor (TGF)- $\beta$  ligand concentrations in women with endometriosis.

Stage of endometriosis lesion development	Function of increased TGF- $\beta$ 1 in endometriosis lesion development	Species	Reference
Immune cell activity	Increased macrophage proliferation	Human	Dou et al., 1997
	Increased macrophage recruitment	Mouse	Hull et al., 2012
	Decreased natural killer cell activity	Human	Mizumoto et al., 1996
Cell survival	Increase in anti-apoptotic factors in eutopic endometrial tissue	Human	Johnson et al., 2005
	Increase in anti-apoptotic factors in ectopic endometrial tissue	Human, mouse	Seoane et al., 2006
	Changes to ectopic endometrial cell metabolism linked to apoptosis resistance	Human	Young et al., 2014a
Cell attachment	Increased attachment of endometrial cells to mouse peritoneal tissue	Human	Beliard et al., 2003
	Increased attachment of endometrial epithelial cells to peritoneal cells	Human	Liu et al., 2009
Cell invasion	Increased invasion of endometrial epithelial cells through peritoneal mesothelial cells	Human	Liu et al., 2009
	Disruption of the peritoneal mesothelial cell monolayer, allowing for ectopic cell invasion	Human	Dunselman et al., 2001; Demir et al., 2004
	Changes to ectopic endometrial cell metabolism linked to increased cell invasion	Human	Young et al., 2014a
Angiogenesis	Increased expression of angiogenic factors from the peritoneal mesothelium	Human	Young et al., 2015.

**Table 2.** Functions that an increase in TGF- $\beta$ 1 has been associated with in the development of peritoneal endometriosis lesions.