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ORIGINAL RESEARCH

Connexin expression pattern in the endometrium of baboons is influenced by hormonal changes and the presence of endometriotic lesions

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ABSTRACT: Experimentally induced endometriosis in baboons serves as an elegant model to discriminate between endometrial genes which are primarily associated with normal endometrial function and those that are changed by the presence of endometriotic lesions. Since connexin genes are characteristic of the hormonally regulated differentiation of the endometrium, we have examined connexin expression in baboon endometrium to delineate if they are altered in response to the presence of endometriotic lesions. Connexin expression in the endometrium of cycling baboons is similar to that of the human endometrium with Connexin(Cx)43 being primarily seen in the stromal compartment and Cx26 and Cx32 being present predominantly in the epithelium. Although Cx32 is up-regulated during the secretory phase, Cx26 and Cx43 are down-regulated. In the baboon model of induced endometriosis a change in connexin pattern was evident in the presence of endometriotic lesions. In the secretory phase, Cx26 and Cx32 are no longer present in the epithelium but Cx26 is now observed primarily in the stromal cells. Infusion of chorionic gonadotrophin in a manner that mimics blastocyst transit *in utero* failed to rescue the aberrant stromal expression of Cx26 that is associated with the presence of endometriotic lesions suggesting an impairment of the implantation process. The altered connexin pattern coupled with a loss of the channel protein in the epithelium and a gain of Cx26 in the stromal compartment suggests that the presence of lesions changes the uterine environment and thereby the differentiation programme. This aberrant expression of connexins may be an additional factor that contributes to endometriosis-associated infertility.

Key words: baboon / chorionic gonadotrophin / connexin26 / connexin43 / endometriosis

Introduction

Endometriosis-associated subfertility is probably due to an altered genetic programme of the eutopic endometrium at the time of uterine receptivity. For successful implantation an activated blastocyst has to interact with the receptive endometrium. For this specific cross talk a number of molecular changes are required at a specific time termed the 'window of implantation'. The genetic programming of the endometrium into the receptive phase is under the control of ovarian steroid hormones and is further modulated by embryonic signals during implantation (Dey *et al*., 2004). In recent years several global gene array analyses have contributed to identifying genes and signal cascades involved in this transformation, however, there still is no satisfying concept of the temporal gene expression pattern that definitely identifies the receptive status of the endometrium in different species (Reese *et al*., 2001; Kao *et al*., 2003; Giudice, 2004; Horcajadas *et al*., 2007). Furthermore, genes whose dysregulation may be associated with or responsible for endometrial changes as a result of gynaecological diseases such as endometriosis are still under investigation (Giudice *et al*., 2002; Wu *et al*., 2006). Among the genes thought to be involved in the synchrony between the embryo and the endometrium we have focused on the role of gap junction connexins (Cxs) in the endometrium of the receptive phase as well as during embryo implantation and in endometriosis (Grümmer and Winterhager, 2008).

Up to now 20 connexin genes have been identified in the mouse and 21 in the human genome (Soehl and Willecke, 2003). The different gap junction channels are characterized by selective permeability

(Mese *et al*., 2007) and have been shown to transfer small interference RNAs (Valiunas *et al*., 2005). The C-terminus of the connexins is subject to post-translational modification and exhibits binding properties to numerous cytoplasmic proteins and thus interacting with intracellular signal cascades (Giepmans, 2004). In rodents suppression of gap junction Cxs26 and 43 precede the receptive phase of the endometrium followed by a local induction of both connexins in the implantation chamber by the embryo (Winterhager *et al*., 1993). This connexin gene expression pattern in the endometrium is regulated via two distinct signalling pathways. During preimplantation, transcription of connexins can be induced by estrogen via an estrogen receptor (ER)-dependent pathway. In contrast, the embryonic/decidual-associated induction of Cx26 and Cx43 utilizes an ER-independent signalling pathway (Grümmer *et al*., 2004; Grümmer and Winterhager, 2008). The role of gap junction communication for decidualization in mice has been shown by Laws *et al*. (2008) using a conditional deletion of the Cx43 gene which leads to impaired decidual cell differentiation in addition to a reduced secretion of several key angiogenic factors.

Corresponding to these findings in the rodent, expression of Cx26 in the epithelium and Cx43 in the stromal cell compartment changes during menstrual cycle in women (Jahn *et al*., 1995; Granot *et al*., 2000). In this context, Cx43 and Cx32 seem to play an additional role in the epithelium, since Cx32 is expressed in the basal portion of the cells (Jahn *et al*., 1995) predominantly in the early secretory phase and Cx43 is more evident in the late secretory phase (Granot *et al*., 2000). In endometriosis, the ectopic lesions reveal a connexin pattern different from the healthy endometrium: the glandular epithelial cells exhibit predominantly strong Cx43 expression whereas Cx26 expression is largely absent (Regidor *et al*., 1997). The aberrant expression of connexins in endometrial diseases indicates that connexins represent a sensitive marker for the physiological state of this tissue and their dysregulation could be associated with pathologies like endometriosis. Here, we describe the expression pattern of connexin transcripts and protein in the endometrium of normally cycling baboons and the alteration in their expression in the endometrium of baboons with experimentally induced endometriosis during the menstrual cycle and following stimulation with chorionic gonadotrophin (CG) to simulate the early events of pregnancy (Fazleabas *et al*., 1999, 2002).

Materials and Methods

Induction of endometriosis

Normally cycling female baboons ranging in age from 7 to 12 years and weighing between 12 and 18 kg were used. The females were housed in individual cages in the Biological Research Laboratory of the University of Illinois (Chicago, IL, USA). All experimental procedures were approved by the Animal Care Committee of the University of Illinois. In this study, a total of 38 female baboons were studied. Endometrial tissues were obtained from 25 disease-free baboons during the proliferative ($n = 8$), and secretory (Days 9–11 post-ovulation [PO]; $n = 11$) phases of the cycle and following CG stimulation during the mid-secretory phase (Days 9–11 PO; $n = 6$). Furthermore, tissues of baboons with induced endometriosis ($n = 8$) in addition to disease-induced baboons treated with CG ($n = 3$) and baboons who developed spontaneous endometriosis ($n = 2$) were analysed. Samples from animals with endometriosis were obtained between Days 9 and 11 PO (Hastings and Fazleabas, 2006). The day of ovulation was monitored by measuring serum estradiol and the day of ovulation was designated as 48 h following the estradiol (E_2) surge (Fazleabas *et al*., 1999).

Endometriosis was induced experimentally in baboons by intraperitoneal inoculation with menstrual endometrium obtained on either Day 1 or 2 of visible menses on two consecutive menstrual cycles. Details of this inoculation have been previously described (Fazleabas *et al*., 2003). Animals treated with CG were fitted with a polyvinyl cannula into the oviduct which permitted infusion of CG into the uterine lumen via an Alzet minipump (Fazleabas *et al*., 1999).

Uterine tissue was obtained at laparotomy from both control animals and baboons with endometriosis and processed for RNA extraction or immunohistochemistry.

RNA preparation, cDNA synthesis and quantitative real-time PCR

Frozen tissues were homogenized and RNA extracted using the Trizol reagent (Molecular Research Center) according to the manufacturer's protocol. RNA quality was determined by photometric analysis and by gel electrophoresis. Following a DNase treatment (Invitrogen), reverse transcription reactions were carried out from 2 μg of total RNA. Subsequent real-time PCR reactions were processed in triplicate using an ABI Prism 7700 Sequence Detector (Applied Biosystems) in a total volume of 25 μl containing 80 ng cDNA, gene-specific primers, and Master Mix including SYBR Green reagent (Applied Biosystems). Quantification was performed by normalizing the expression levels of the genes analysed to the housekeeping gene beta-actin (ACTB). The following primers were used: cx26-forward 5′-ATCTTCTTCCGGGTCGTCTT-3′, Cx26-reverse 5′-GACACCGCAATCATGAACAC-3′ (product size: 179 bp), Cx43-forward 5′-TGGATTCAGCTTGAGTGCTG-3′, Cx43-reverse 5′-CAAG GGCGTTAAGGATCGGG-3′ (product size: 188 bp), Cx32-forward 5′-CTGCTCTACCCTGGCTATGC-3′, Cx32-reverse 5′-CCACATTGAG GATGATGCAG-3′ (product size. 157 bp), ACTB-forward 5′-ACCAAC TGGGACGACATGGAGAAAA-3′, ACTB-reverse 5′-TACGGCCAGAG GCGTACAGGGATAG-3′ (product size: 213 bp). Melting curve analysis allowed determination of specificity of the PCR fragments. The specificity of the primers for baboon has been proven for liver (Cx26, Cx32) and heart tissue (Cx43), respectively (data not shown).

Immunohistochemistry

Tissues were frozen in Neg 50 Frozen Section Medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Five micron sections were cut on a Microtome HM500 OM cryostat (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Cx26, Cx32 and Cx43 were detected using indirect immunofluorescence. Frozen sections were washed with PBS and fixed in ice cold absolute ethanol for 10 min. Immunoincubation was performed as described previously (Winterhager *et al*., 1991). Antibodies used were anti-Cx26 rabbit polyclonal antibody and anti-Cx32 rabbit polyclonal antibody (1 : 150, both Zymed Laboratories, San Francisco, CA, USA), and mouse monoclonal anti-Cx43 antibody (1 : 100; Zymed Laboratories San Francisco, CA, USA). For cytokeratin staining, a mouse monoclonal antibody was used (1 : 1, Becton Dickinson, San Jose, CA, USA). Double immunolabelling was performed as described elsewhere (Gellhaus *et al*., 2004). An appropriate FITC or Alexa-conjugated secondary antibody (DAKO) was used. For controls the primary antibody was omitted and positive controls were performed on different baboon tissues from a pool of untreated animals. Cx26 and Cx32 were tested on liver, Cx40 and Cx43 and Cx37 (endothelial cells) on heart, and Cx31 on skin sections. Staining was analysed with a Nikon E400 microscope and the images were captured using Spot 4.1 colour cooled digital camera equipped with advanced image capture software (Diagnostic Instruments,

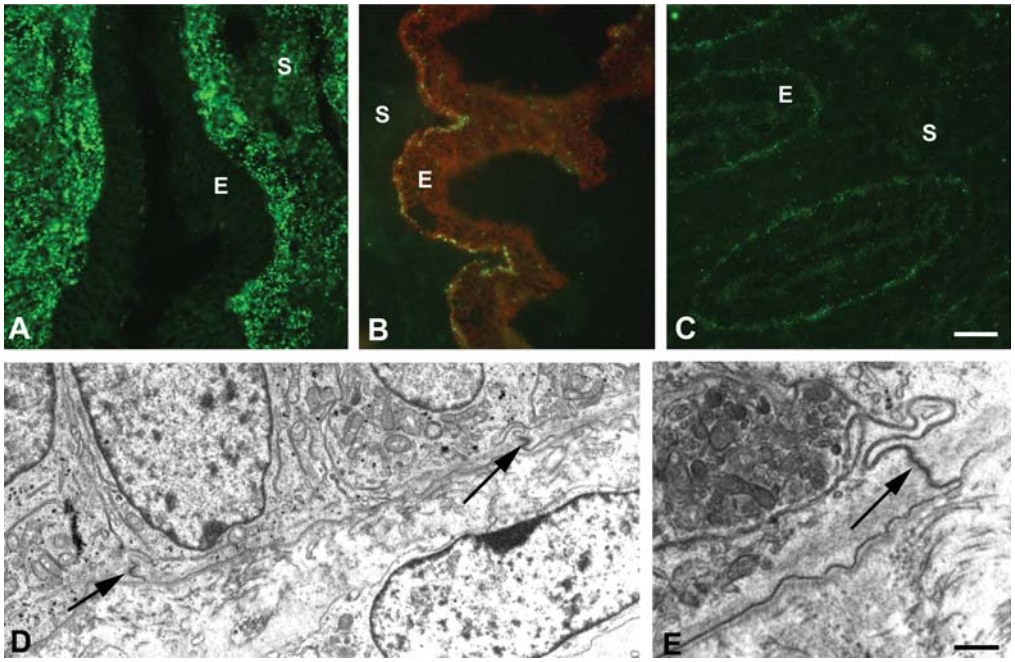

Figure 1 (**A**–**C**) Immunohistochemistry of baboon endometrium in the proliferative phase.

Cx43 is strongly expressed in the stromal compartment of the endometrium whereas the epithelium lacks any immunoreaction (A), double-immunolabelling of Cx26 and cytokeratin revealed punctuate reaction to Cx26 in the outermost basal part of the uterine epithelium at the border to the stroma (B). Immunoreaction to Cx32 in the epithelium is weaker but like Cx26 at the basal portion between the cells (C). Bar = 100 μm. (**D** and **E**) Electron microscopic investigations confirmed junctional structures (arrows) located basally between neighbouring epithelial cells (D). Higher magnification showed gap junctional structures (arrow) (E). E, epithelium; S, stroma. Bar = 2 μm in D, 0,4 μm in E.

Sterling Heights, MI, USA) at magnification of 40×. A minimum of six sections per animal were analysed.

Electron microscopy

Endometrial tissues were fixed in 3% (w/v) paraformaldehyde/1% (v/v) glutaraldehyde by immersion for 6 h at room temperature and subsequently post-fixed in 1% osmium tetroxide (v/v)/1.5% potassium ferrocyanide (w/v) in distilled water for 1 h at room temperature, and all tissues were processed into Araldite epoxy resin (Ladd Research Industries, Burlington, VT, USA). For ultrastructural examination, sections (70–90 nm) were contrasted with uranyl acetate/lead citrate before examination with a Philips 301 or CM10 electron microscope.

Results

Immunohistochemical analysis for connexins Cx26, Cx31, Cx32, Cx37 and Cx40 revealed only the presence of Cx26, Cx32 and Cx43 in baboon endometrium.

Connexin staining during the menstrual cycle

Immunolocalization of Cx43 in cycling baboons showed a strong and typical punctuate staining in the stromal compartment during the entire proliferative phase whereas the epithelium lacked any immunoreactivity (Fig. 1A). In contrast, Cx26 was only detected in the uterine epithelium, however, in an unusual and untypical localization of the epithelial compartment. Cx26 is clearly located basally at the cell membranes forming a pearl chain like row (Fig.1B) This staining pattern remained constant throughout the proliferative phase. Co-immunostaining with cytokeratin confirmed that the Cx26 gap junctions still belong to the epithelial compartment (Fig. 1B). In addition to Cx26, Cx32 is found in the uterine epithelium and like Cx26 predominantly located in the basal region of the epithelium, however, not in such a confined manner (Fig. 1C).

The presence of gap junctions at the very outermost basal portion of the uterine epithelial cells during the proliferative phase is confirmed by electron microscopic investigations revealing typical gap junction structures in this membrane area (Fig. 1D and E).

During the secretory phase, stromal Cx43 staining declined (Fig. 2A) and Cx26 was strongly reduced or even absent (Fig.2 B). Cx32 immunostaining, however, was still present in the epithelium, and still clearly defined to the basal portion of the lateral membranes (Fig. 2C).

Connexin staining in the secretory phase: endometriosis

Since the endometrial biopsies of baboons with induced endometriosis were biopsied in the secretory phase, the expression pattern of connexins were compared with the same cyclic phase of healthy animals. Between 10 and 15 month after inoculation of the menstrual tissue, the connexin distribution pattern in the eutopic endometrium of baboons with induced disease was changed for Cx26 and Cx32 whereas no obvious alteration in Cx43 localization in the stromal compartment was observed, although the intensity of staining was

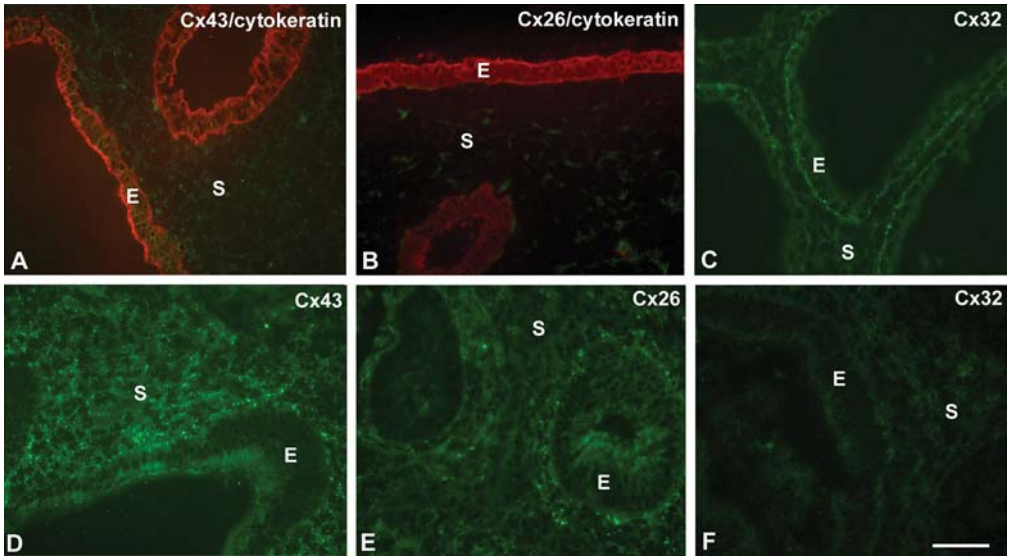

Figure 2 (**A**–**C**) Immunohistochemistry of baboon endometrium in the secretory phase.

Double immunolabelling of Cx43 and cytokeration revealed a weak staining for Cx43 in the stromal compartment (A) and no staining for Cx26 (B). A clear and intense immunoreaction to Cx32 is found at the basal portion of the uterine epithelium (C). Bar=100 μm. (**D**–**F**) Immunohistochemistry of baboon endometrium in the secretory phase with induced endometriosis (10–15 month). Staining of Cx43 is seen in the stromal compartment (D). Cx26 is clearly expressed in the stromal compartment but not in the epithelium (E). There is no immunoreaction to Cx32 (F). E, epithelium; S, stroma. Bar = 100 μm.

enhanced compared with controls (Fig. 2D compared with 2A). Cx26 protein completely changed its localization and was now detected in the stromal compartment instead of in the epithelium. This switch from the epithelial to the stromal compartment was accompanied with a higher intensity of Cx26 staining (Fig. 2E). Staining for Cx32 was quite weak in the epithelial compartment but unlike Cx26 there was no switch in staining patterns from the epithelial to the stromal compartment (Fig. 2F).

Connexin pattern after CG infusion with or without induced endometriosis

The infusion of CG which led to the typical plaque reaction of the epithelium revealed a clear expression of Cx43 protein in the stromal compartment (Fig. 3A), however, staining for Cx26 or Cx32 was absent in the epithelium (Fig. 3B and C). Interestingly, infusion of CG in to animals with prolonged induced endometriosis (10–15 month) showed the same pattern of staining as was observed in the secretory phase of cycling baboons with endometriosis: no changes in Cx43 distribution but a distinct switch in staining for Cx26 detectable in the stromal compartment, and mostly no Cx32 protein expression. However, in some sections Cx32 staining can be found in restricted stromal areas without preferential localization (Fig. 3D–F).

Transcript levels of Cxs26, 32, 43

The presence and intensity of the connexin immunostaining is mostly reflected in transcript levels using qPCR. Similarly to the intensity of immunolabelling, transcript levels of Cx32 were significantly lower when compared with those of Cx43 and Cx26 (Fig. 4A–C).

Furthermore corresponding to protein expression, Cx26 and Cx43 were down-regulated in the secretory phase (Fig. 4A and B), whereas an increase of Cx32 transcript was observed (Fig. 4C). In addition, the presence of endometriotic lesions the slight increase in Cx26 could be confirmed together with the further down-regulation of Cx32 mRNA (Fig. 4A and C). In contrast, the decrease of Cx43 transcript levels were not reflected in the staining intensity of the Cx43 protein (Fig. 4B).

Lower levels of mRNA were found for all connexins after CG treatment (Fig. 4) mirrored the results from immunostaining for Cx26 and Cx32, however, again the relatively high amount of Cx43 protein in the stromal compartment is not reflected by transcript levels. Infusion of CG into animals with disease did not seem to influence the connexin expression levels. In summary Cx26 and Cx32 staining levels correlated to the levels of transcripts whereas Cx43 immunostaining was higher in the stromal compartment and was unrelated to transcript levels.

Discussion

Several studies have shown that the proposed molecular markers of a receptive endometrium are aberrantly expressed in women with endometriosis (reviewed in Giudice *et al.*, 2002). Genome-wide microarray comparisons between women with or without endometriosis have further validated the concept that endometrial gene expression within the window of uterine receptivity is altered (Kao *et al.*, 2003; Absenger *et al.*, 2004). However, it is still a problem to discriminate if changes in the gene pattern for endometrial receptivity are the cause for this disease, or if these changes are a consequence of the presence of endometriotic lesions. Using an induced endometriosis model in baboons we here added an additional marker molecule which is aberrantly expressed upon long-term presence

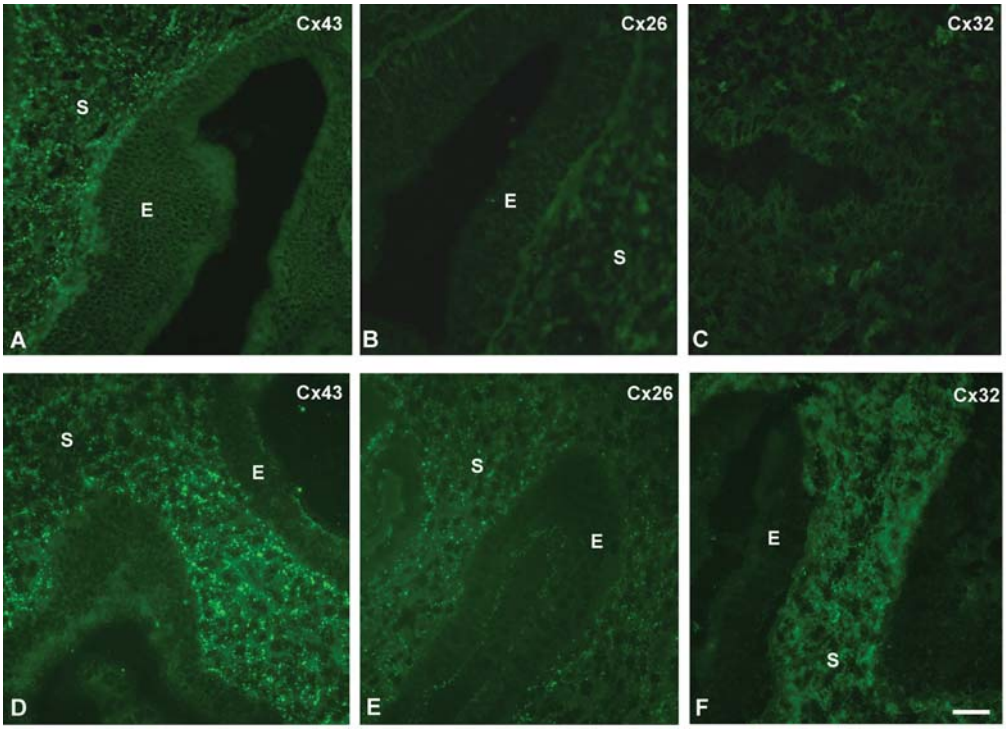

Figure 3 (**A**–**C**) Immunohistochemistry of baboon endometrium after CG infusion. Cx43 is expressed in the stromal compartment but not in the epithelium showing the plaque reaction (A). There is neither an immunoreaction to Cx26 (B) nor to Cx32 (C). (**D**–**E**) Immunohistochemistry of baboon endometrium after CG infusion and induced endometriosis (10–15 month). There is no obvious change of Cx43 immunostaining in the stromal compartment (D). Cx26 is expressed in the stromal compartment (E), Cx32 is not detected (F). E, epithelium; S, stroma. Bar = 100 μm.

(>10 month) of endometriotic lesions. Our results further confirm the hypothesis that the altered endometrial program is in part due to the presence of lesions. Since the alteration occurred during the secretory phase these changes could explain the problem of impaired fertility associated with endometriosis.

Gap junction connexin proteins are precisely regulated during the menstrual cycle in non-human primates. Similarly to findings in human endometrium (Jahn *et al*., 1995) we found Cx43 in the stromal and Cx26 in the epithelial compartment of baboons, both being down-regulated in the secretory phase. Moreover as found in humans, Cx32 is expressed in the baboon epithelium and restricted to the basal membrane region (Jahn *et al*., 1995). In contrast to the human endometrium, both connexins are located at the basal portion where gap junction structures are seen by electron microscopy. The reason for this very defined basal distribution of Cx26 and Cx32 is not readily discernable from this study.

The paracrine secretion of baboon trophoblast CG mimicked by CG infusion influenced this pattern and resulted in a disappearance of Cx26 and Cx32 from the epithelium which underwent the typical plaque reaction. This pattern of connexin regulation is comparable with the situation in rodents at the time of uterine receptivity (Winterhager *et al*., 1993) with a loss of epithelial connexins prior to implantation. In the presence of endometriotic lesions, Cx26 switched from the epithelium to the stromal compartment in the secretory phase which points to an inappropriate differentiation of the stromal fibroblasts at receptivity. Interestingly, this change in Cx26 pattern persisted in baboons with endometriosis even following CG infusion.

The crucial role for Cx43 in decidualization and angiogenesis during implantation has been shown in mice with tissue specific deletion using Cre-recombinase under the control of the progesterone promoter (Laws *et al*., 2008). The Cx43 protein pattern does not appear to be influenced by endometriosis; however, a reduction in the level of mRNA transcripts is readily evident. At present, we cannot explain the discrepancy between transcript levels and immunostaining of Cx43, however, we can only speculate that this discrepancy could be related to changes in connexin protein turnover due to altered phosphorylation of the C-terminus (Solan and Lampe, 2009) or a shift in the ratio of the stromal to the epithelial compartment.

These changes in endometrial connexin patterns as a result of the establishment of endometriotic lesions support previous studies for several other marker genes. The CCN1 (CYR61) protein is an estrogen-regulated proangiogenic factor which is aberrantly expressed in human endometriotic eutopic endometrium during the window of receptivity (Absenger *et al*., 2004) and is highly up-regulated in eutopic endometruim upon induction of endometriosis in baboons (Gashaw *et al*., 2006). Another candidate, cFOS (c-fos), revealed altered levels and distribution in the eutopic endometrium of baboons after the induction of endometriosis (Hastings *et al*., 2006). All three genes, Cx26, CYR61 and c-fos are estrogen regulated genes which are normally suppressed under progesterone dominance.

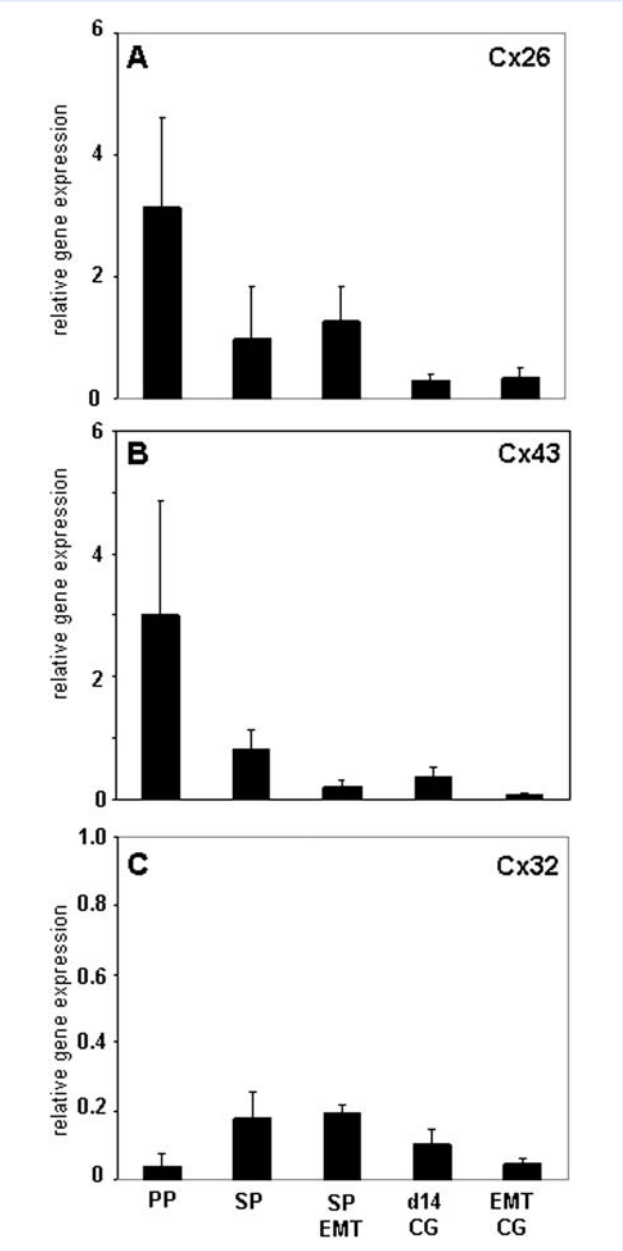

Figure 4 Quantitative Real-Time RT–PCR analysis of transcripts of Cx26 (**A**), Cx43 (**B**) and Cx32 (**C**) in endometrium of the proliferative phase (PP), and secretory phase (SP) of healthy animals, and secretory phase of induced endometriosis (SP EMT).

In addition, endometrium of healthy animals (d14 CG) as well as of animals with induced endometriosis (EMT CG) was analysed after 14 days of treatment with chorion gonadotrophin.

The HOXA10 protein which is crucial for uterine receptivity was significantly decreased after 12 and 16 months of induced disease in both the epithelial and stromal cells of the endometrium (Kim *et al.*, 2007). In contrast to Cx26, CYR61 and c-fos, hormonal regulation of HOXA10 in adult uteri is dependent on both estrogen and progesterone. The increase in HOXA10 mRNA levels, however, is higher in response to the progestin medroxyprogesterone acetate (MPA) than to 17β-estradiol in primary endometrial stromal cells, and a combination of 17β-estradiol and MPA revealed a synergistic action on HOXA10 gene expression levels (reviewed in Daftary and Taylor, 2006). Thus, the down-regulation of HOXA10 seems to point to an impaired response to progesterone together with the methylation of the HOXA 10 promoter (Kim *et al.*, 2007).

Several studies from different groups including ours have provided evidence that secretory-phase eutopic endometrium in women as well as in baboons with endometriosis responds inadequately to progesterone (Wu *et al.*, 2006; Aghajanova *et al.*, 2009a, b; Wang *et al.*, 2009). This is in line with clinical observations that a cohort of endometriosis patients is resistant to progestin treatment (Bulun *et al.*, 2006). Comparative gene expression analysis of progesterone-regulated genes in the receptive endometrium confirmed that the progesterone response is attenuated in the endometrium of patients affected by endometriosis (Burney *et al.*, 2007). The molecular basis of progesterone resistance in endometriosis may be acquired by different mechanisms. In humans it has been reported that the PRA to PRB ratio is down-regulated in endometriotic tissues leading to a relative resistance to progesterone action and an impaired stromal differentiation of eutopic as well as ectopic endometrium in endometriosis (Attia *et al.*, 2000; Igarashi *et al.*, 2005; Wu *et al.*, 2006). In baboons with induced endometriosis hormonal treatment of isolated stromal cells did not increase levels of PRA or PRB in contrast to healthy animals. Furthermore, ERalpha and ERbeta were decreased in the endometrial stromal cells (Jackson *et al.*, 2007). This altered expression of steroid hormone receptors appears to be associated with a reduced response to progesterone in both the eutopic and ectopic endometrium. Thus the modus of aberrant endometrial gene regulation in the presence of endometriotic lesions shows similarities for a number of genes and leads to the hypothesis that it is the presence of the disease that gives rise to the phenomenon of progesterone resistance. Furthermore, CG treatment is not able to restore the impaired connexin distribution. This response is similar to our previous studies, where CG infusion failed to induce expression of the immunoregulatory protein glycodelin or alpha-smooth muscle actin (alpha-SMA) in stromal cells in animals with endometriosis as early as 1 and 4 months following the induction of disease (Fazleabas *et al.*, 2003).

Since alterations in a previously healthy eutopic endometrium occurs as a function of time in the presence of endometriotic lesions, additional factors such as inflammatory cytokines could influence the genetic program. Endometriosis is associated with a pelvic inflammatory process which results in altering the function of immune-related cells and increased number of activated macrophages that secrete factors such as C-Reactive Protein, Serum Amyloid A, tumour-necrosing factor (TNF)-alpha, Monocyte Chemotactic Protein 1, Interleukins (IL)-6, IL-8 and Cognate Chemokine Receptor 1 (Agic *et al.*, 2006). Since Cx26 is known to respond to inflammatory mediators such as IL1 and TNFalpha in a hepatocyte cell line (Temme *et al.*, 1998) we cannot exclude the possibility that the altered inflammatory milieu as a consequence of endometriosis response contributes to this aberrant pattern of Cx26 staining.

Taken together, this study adds further credence to the increasing evidence that the presence of endometriotic lesions has a detrimental effect on the receptive endometrium. The impaired physiological status predominantly in the stromal compartment indicates a crucial role of stromal fibroblasts for endometrial programming and could

contribute to an impaired endometrial gene expression. Because of well-known estrogen dependency of Cx26 the shift in the expression pattern of this connexin could be due to the progesterone resistance that develops in the stromal cells of baboons with endometriosis (Jackson *et al*., 2007; Kim *et al*., 2007).

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References

Absenger Y, Hess-Stumpp H, Kreft B, Krätzschmar J, Haendler B, Schütze N, Regidor PA, Winterhager E. Cyr61, a deregulated gene in endometriosis. *Mol Hum Reprod* 2004;**10**:399–407.

Aghajanova L, Hamilton A, Kwintkiewicz J, Vo KC, Giudice LC. Steroidogenic enzyme and key decidualization marker dysregulation in endometrial stromal cells from women with versus without endometriosis. *Biol Reprod* 2009a;**80**:105–114.

Aghajanova L, Velarde M, Giudice LC. The progesterone receptor Co-activator Hic-5 is involved in the pathophysiology of endometriosis. *Endocrinology* 2009b; Epub ahead of print.

Agic A, Xu H, Finas D, Banz C, Diedrich K, Hornung D. Is endometriosis associated with systemic subclinical inflammation? *Gynecol Obstet Invest* 2006;**62**:136–138.

Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. *J Clin Endocrinol Metab* 2000;**85**:2897–2902.

Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, Innes J, Julie Kim J. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. *Mol Cell Endocrinol* 2006;**248**:94–03.

Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA, Giudice LC. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* 2007;**148**:3814–3826.

Daftary GS, Taylor HS. Endocrine regulation of HOX genes. *Endocr Rev* 2006;**27**:331–355.

Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, Wang H. Molecular cues to implantation. *Endocr Rev* 2004;**25**:341–373.

Fazleabas AT, Donnelly KM, Srinivasan S, Fortman JD, Miller JB. Modulation of the baboon (Papio anubis) uterine endometrium by chorionic gonadotrophin during the period of uterine receptivity. *Proc Natl Acad Sci U S A* 1999;**96**:2543–2548.

Fazleabas AT, Brudney A, Gurates B, Chai D, Bulun SE. A modified baboon model for endometriosis. *Ann N Y Acad Sci* 2002;**955**:308–317.

Fazleabas AT, Brudney A, Chai D, Langoi D, Bulun SE. Steroid receptor and aromatase expression in baboon endometriotic lesions. *Fertil Steril* 2003;**80**:820–827.

Gashaw I, Hastings JM, Jackson KS, Winterhager E, Fazleabas AT. Induced endometriosis in the baboon (Papio anubis) increases the expression of the proangiogenic factor CYR61 (CCN1) in eutopic and ectopic endometria. *Biol Reprod* 2006;**74**:1060–1066.

Gellhaus A, Dong X, Propson S, Maass K, Klein-Hitpass L, Kibschull M, Traub O, Willecke K, Perbal B, Lye SJ *et al*. Connexin43 interacts with NOV: a possible mechanism for negative regulation of cell growth in choriocarcinoma cells. *J Biol Chem* 2004;**279**:36931–36942.

Giepmans BN. Gap junctions and connexin-interacting proteins. *Cardiovasc Res* 2004;**62**:233–245.

Giudice LC. Microarray expression profiling reveals candidate genes for human uterine receptivity. *Am J Pharmacogenomics* 2004;**4**:299–312.

Giudice LC, Telles TL, Lobo S, Kao L, Ann NY. The molecular basis for implantation failure in endometriosis: on the road to discovery. *Ann N Y Acad Sci* 2002;**955**:252–264; discussion 293–295, 396–406.

Granot I, Dekel N, Bechor E, Segal I, Fieldust S, Barash A. Temporal analysis of connexin43 protein and gene expression throughout the menstrual cycle in human endometrium. *Fertil Steril* 2000;**73**:381–386.

Grümmer R, Winterhager E. Connexins: indicators for hormonal and blastocyst-mediated endometrial differentiation. In Aplin JD, Asgerally TF, Glasser S, Giudice LC (eds). *The Endometrium. Molecular, Cellular and Clinical Perspectives*. Informa healthcare UK Ltd, 2008. 319–330.

Grümmer R, Hewitt SW, Traub O, Korach KS, Winterhager E. Different regulatory pathways of endometrial connexin expression: preimplantation hormonal-mediated pathway versus embryo implantation-initiated pathway. *Biol Reprod* 2004;**71**:273–281.

Hastings JM, Fazleabas AT. A baboon model for endometriosis: implications for fertility. *Reprod Biol Endocrinol* 2006;**4**:7.

Hastings JM, Jackson KS, Mavrogianis PA, Fazleabas AT. The estrogen early response gene FOS is altered in a baboon model of endometriosis. *Biol Reprod* 2006;**75**:176–182.

Horcajadas JA, Díaz-Gimeno P, Pellicer A, Simón C. Uterine receptivity and the ramifications of ovarian stimulation on endometrial function. *Semin Reprod Med* 2007;**25**:454–460. Review.

Igarashi TM, Bruner-Tran KL, Yeaman GR, Lessey BA, Edwards DP, Eisenberg E, Osteen KG. Reduced expression of progesterone receptor-B in the endometrium of women with endometriosis and in cocultures of endometrial cells exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. *Fertil Steril* 2005;**84**:67–74.

Jackson KS, Brudney A, Hastings JM, Mavrogianis PA, Kim JJ, Fazleabas AT. The altered distribution of the steroid hormone receptors and the chaperone immunophilin FKBP52 in a baboon model of endometriosis is associated with progesterone resistance during the window of uterine receptivity. *Reprod Sci* 2007;**14**:137–150.

Jahn E, Classen-Linke I, Kusche M, Beier HM, Traub O, Grümmer R, Winterhager E. Expression of gap junction connexins in the human endometrium throughout the menstrual cycle. *Hum Reprod* 1995;**10**:2666–2670.

Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* 2003;**144**:2870–2881.

Kim JJ, Taylor HS, Lu Z, Ladhani O, Hastings JM, Jackson KS, Wu Y, Guo SW, Fazleabas AT. Altered expression of HOXA10 in endometriosis: potential role in decidualization. *Mol Hum Reprod* 2007;**13**:323–332.

Laws MJ, Taylor RN, Sidell N, DeMayo FJ, Lydon JP, Gutstein DE, Bagchi MK, Bagchi IC. Gap junction communication between uterine stromal cells plays a critical role in pregnancy-associated neovascularization and embryo survival. *Development* 2008;**135**:2659–2668.

Mese G, Richard G, White TW. Gap junctions: basic structure and function. *J Invest Dermatol* 2007;**127**:2516–2524.

Reese J, Zhao X, Ma WG, Brown N, Maziasz TJ, Dey SK. Comparative analysis of pharmacologic and/or genetic disruption of cyclooxygenase-1 and cyclooxygenase2 function in female reproduction in mice. *Endocrinology* 2001;**143**:3198–3206.

Regidor PA, Regidor M, Schindler AE, Winterhager E. Aberrant expression pattern of gap junction connexins in endometriotic tissues. *Mol Hum Reprod* 1997;**3**:375–381.

Soehl G, Willecke K. An update on connexion genes and their nomenclature in mouse and man. *Cell Commun Adhes* 2003;**10**:173–180.

Solan JL, Lampe PD. Connexin43 phosphorylation: structural changes and biological effects. *Biochem J* 2009;**419**:261–272.

Temme A, Traub O, Willecke K. Downregulation of connexin32 protein and gap-junctional intercellular communication by cytokine-mediated acute-phase response in immortalized mouse hepatocytes. *Cell Tissue Res* 1998;**294**:345–350.

Valiunas V, Polosina YY, Miller H, Potapova IA, Valiuniene L, Doronin S, Mathias RT, Robinson RB, Rosen MR, Cohen IS *et al.* Connexin-specific cell-to-cell transfer of short interfering RNA by gap junctions. *J Physiol* 2005;**568**:459–468.

Wang C, Mavrogianis PA, Fazleabas AT. Endometriosis is associated with progesterone resistance in the baboon (papio anubis) oviduct: evidence based on the localization of oviductal glycoprotein 1 (OVGP1). *Biol Reprod* 2009;**80**:272–278.

Winterhager E, Stutenkemper R, Traub O, Beyer E, Willecke K. Expression of different connexin genes in rat uterus during decidualization and at term. *Eur J Cell Biol* 1991;**55**:133–142.

Winterhager E, Grümmer R, Jahn E, Willecke K, Traub O. Spatial and temporal expression of connexin26 and connexin43 in rat endometrium during trophoblast invasion. *Dev Biol* 1993;**157**: 399–409.

Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. *Epigenetics* 2006;**2**:106–111.

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