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Research Article

Cigarette smoking alters sialylation in the Fallopian tube of women, with implications for the pathogenesis of ectopic pregnancy†

Short title: Sialylation and ectopic pregnancy in women

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Key words: sialic acid, sialyltransferases, tubal ectopic pregnancy, cigarette smoking, cilia, galectin

Abbreviations: Galβ1,3/4GlcNAc, galactoseβ1, 3/4*N*-acetylglucosamine (lactosamine); MAM, *Maackia amurensis*; SNA, *Sambucus sieboldiana*; ST3GAL, α2,3-galactoside sialyltransferases; ST6GAL, α2,6-galactoside sialyltransferases.

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Abstract

Sialylation creates a negative charge on the cell surface that can interfere with blastocyst implantation. For example, α2,6-sialylation on terminal galactose, catalyzed by the sialyltransferase ST6GAL1, inhibits the binding of galectin-1, a *β*-galactoside-binding lectin. We recently reported the potential involvement of galectin-1 and -3 in the pathogenesis of tubal ectopic pregnancy; however, the precise role of galectins and their ligand glycoconjugates remain unclear. Here, we investigated the expression of the genes encoding α2,3- and α2,6-galactoside sialyltransferases (*ST3GAL1−6* and *ST6GAL1−2*) and the localization of sialic acids in the Fallopian tube of women with or without ectopic implantation. *ST6GAL1* expression was higher in the mid-secretory phase than the proliferative phase of non-pregnant women ($P<0.0001$), whereas *ST6GAL1* ($P<0.0001$), *ST3GAL3* ($P=0.0029$), *ST3GAL5* ($P=0.0089$), and *ST3GAL6* ($P=0.0018$) were all lower in Fallopian tubes with ectopic implantations. Both α2,3- and α2,6-sialic acids, however, remained enriched on the surface of Fallopian tube epithelium. Cigarette smoking, a major risk factor for tubal ectopic pregnancy, was associated with reduced mid-secretory-phase expression of *ST6GAL1* ($P=0.0298$), but elevated expression of *ST3GAL5* ($P=0.0006$), an enzyme known to be involved in ciliogenesis. Indeed, sialic acid-containing ciliated inclusion cysts, which are associated with abnormal ciliogenesis, were observed within the epithelium at a higher frequency in women who smoked ($P=0.0177$), suggesting that abnormal ciliogenesis is associated with smoking. Thus, cigarette smoking alters sialylation in the Fallopian tube epithelium, and is potentially a source of decreased tubal transport and increased receptivity for blastocyst in the human Fallopian tube. This article is protected by copyright. All rights reserved

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Introduction

Ectopic pregnancy occurs when a blastocyst implants outside the uterus. Ectopic implantation occurs in 2% of all pregnancies, and remains one of the leading causes of maternal death in the first trimester (Farquhar, 2005; Varma and Gupta, 2012); over 95% of these implantations occurs in the Fallopian tube (Walker 2007; Varma and Gupta 2012). Although the pathogenesis of tubal ectopic pregnancy is still unclear, risk factors include reduced or impaired tubal transport activity and/or increased tubal receptivity for blastocyst implantation; tubal damage as a result of surgery or infection; cigarette smoking; and in vitro fertilization (reviewed by Shaw et al, 2010).

Apposition of the blastocyst to the epithelium during implantation involves the interaction of glycosylated proteins, lipids, and mucins. In rabbits, the uterine luminal epithelium undergoes extensive differentiation, accompanied by a loss of negative charge on the cell surface prior to blastocyst implantation (Anderson and Hoffman, 1984); a similar change in net surface charge as well as alterations to the glycocalyx have been noted prior to implantation in rats and mice (Hewitt et al, 1979; Chávez and Anderson, 1985). Sialic acids seem to be a major contributor to the cell surface negative charge since treatment with neuraminidase, which hydrolyses sialic acid residues, replicates the phenotype pre-implantation (Jenkinson and Searle, 1977).

Sialic acids are a class of negatively charged acidic sugars with a common nine-carbon carboxylated backbone. Sialic acids often occupy the terminal position of cell surface glycoproteins and glycolipids – an exposed location that influences oligosaccharide structure and allows them to mediate a variety of biological phenomena, such as cell-cell interaction, cell migration, adhesion, and

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to regulate pathological events, such as inflammation and cancer metastasis. A family of glycosyltransferases, called sialyltransferases, catalyzes the addition of sialic acid to an acceptor carbohydrate. The enzymes that transfer sialic acid to the terminal galactose residues of glycochains are classified into two groups according to the linkage of the catalyzed sialic acid: β-galactoside α2,3-sialyltansferases (ST3GAL) and β-galactoside α2,6-sialyltransferases (ST6GAL); six members belong to the ST3GAL family and two members belong to the ST6GAL family in mice and humans (Harduin-Lepers et al, 2001).

Sialic acid abundance in the endometrium decreases at the time of implantation in rats and rabbits (Rajalakshmi et al, 1972; Anderson et al, 1986), and sialylation is reduced in the decidua of early pregnancy in women (Jones et al, 2010). Conversely, an enrichment of sialic acids seems to delay implantation in rats (Sankaran and Prasad, 1973), suggesting the involvement of sialic acids during normal blastocyst implantation. Yet, little is known about the role of glycoconjugates, including sialic acids, in the ectopic implantation of blastocysts in the Fallopian tube.

Galectins are endogenous lectins that bind to β-galactoside sugar residues on the cell surface, and possess a high affinity for galactose β-linked to *N*-acetyglucosamine (*N*-acetyllactosamine). α2,6-sialylation on a terminal galactose inhibits galectin-1 binding, whereas sialyation has no effect on the binding of galectin-3. We recently reported that the expression and localization of galectin-1 and galectin-3 are altered (galectin-1 increases whereas galectin-3 decreases) in the Fallopian tube of women with ectopic implantation (Nio-Kobayashi et al, 2015). This relationship suggests the involvement of lectin-glycoconjugate interactions, especially galectin-1-glycoconjugate binding,

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during the pathogenesis of ectopic implantation – although the glycoconjugate targets for galectins and their functions during normal and pathological implantation are still unclear.

Antecedents in women at increased risk of tubal ectopic pregnancy led us to hypothesize that glycoconjugates are altered in tubal ectopic pregnancy. We investigated the function of sialic acids during normal tubal function and ectopic implantation by assessing the expression of *ST3GAL1−6* and *ST6GAL1−2* and the localization of α2,3- or α2,6-sialic acids in Fallopian tubes collected from non-pregnant women across the menstrual cycle and from women with tubal ectopic pregnancy. We further examined the Fallopian tubes of women with increased risk for ectopic pregnancy, revealing the effects of cigarette smoking on the expression of sialyltransferases and sialic acid abundance.

Results

Expression of enzymes involved in sialylation in the Fallopian tube

Transcripts encoding both *β*-galactoside α2,3-sialyltransferases (*ST3GAL1−6*) and *β*-galactoside α2,6-sialyltransferases (*ST6GAL1−2*) are present in the Fallopian tubes of non-pregnant women, in both the proliferative and secretory phases of the menstrual cycle (Table 1). Transcript abundance of *ST6GAL1* was higher during the mid-secretory phase compared to the proliferative phase ($P<0.0001$). Fallopian tubes from women with ectopic implantations, however, had significantly less *ST6GAL1* transcript ($P<0.0001$). Women with tubal ectopic pregnancy also exhibited reduced expression of *ST3GAL3* ($P=0.0029$), *ST3GAL5* ($P=0.0089$), and *ST3GAL6* ($P=0.0018$) in the Fallopian tube compared to non-pregnant women during the mid-secretory phase

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(Table 1).

Influence of cigarette smoking on the expression of β-galactoside sialyltransferases in the Fallopian tube

We next examined if cigarette smoking, a key risk factor for tubal ectopic pregnancy, affects the expression of sialyltransferases in the human Fallopian tube. The relative gene expression compared among all samples (in mixed menstrual cycles, to increase the cohort size; n=14 for confirmed non-smokers versus n=6 for confirmed smokers) revealed significantly more *ST3GAL5* transcript in smokers (P=0.0169) (Table 2). This difference between smokers and non-smokers was also observed specifically during the mid-secretory phase (P=0.0006) (Fig. 1A). Given that *ST6GAL1* expression changes during the menstrual phase, we restricted our comparison to either the proliferative phase or mid-secretory phase. Despite the small number of confirmed smokers (n=3 for proliferative or mid-secretory phases), the expression of *ST6GAL1* in the mid-secretory phase was significantly lower in the Fallopian tubes of confirmed smokers (P=0.0298) (Fig.1B).

Localization of sialic acids in the Fallopian tube

Lectin histochemistry using α2,3- or α2,6-sialic acid-specific plant lectins was performed to examine sialylation within the human Fallopian tube. Examination of both MAM (from *Maackia amurensis*; binds α2,3-sialic acids) and SNA (from *Sambucus sieboldiana*; binds α2,6-sialic acids) revealed abundant and selective localization of both sialic acids, which are catalyzed by the

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sialyltransferases ST3GAL and ST6GAL families (Fig. 2). Intense reactivity for both MAM and SNA was found in the stroma of the Fallopian tubes (Fig. 2A-B). Both α2,3- and α2,6-sialic acids also localized to the apical region of the epithelium (Fig. 2C-D), particularly along the cilia (Fig. 2E-F). Non-ciliated secretory cells labeled by MAM and SNA in various intensities and patterns (Fig. 2E-F). α2,3-sialic acids were largely restricted to the apical surface of epithelium (Fig. 2E) whereas α2,6-sialic acids were found at the surface as well as in vesicular structures in the cytoplasm of both types of ciliated and non-ciliated cells (Fig. 2F). These patterns were consistent in the Fallopian tubes of proliferative and secretory phases of the menstrual cycle and of ectopic pregnancies. Sialylation is therefore enriched in the cilia along the luminal surface of Fallopian tube epithelial cells.

Formation of sialic acid-containing ciliated cysts in the Fallopian tube epithelium

Although both α2,3- and α2,6-sialic acids were generally restricted to the apical epithelial surface, the sialic acid-positive spherical structures sporadically observed within the Fallopian tube epithelium (Fig. 3A-B) were identified as "ciliated cysts", which are structures associated with abnormal ciliogenesis. Careful observation of the MAM- or SNA-stained sections revealed that α2,3- and α2,6-sialic acids concentrated at the concave base of cilia (Fig. 3C) and stained ciliary structures with a jug-like shape just below the luminal surface of the epithelium (Fig. 3D). Stained cilia also formed round cysts that were completely buried into the epithelium in the cytoplasm of some cells (Fig. 3E), and could sometimes be found at the bottom of the epithelium with neighboring elongated nuclei (Fig. 3F).

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Although ciliated cysts containing α2,3- and α2,6-sialic acids were observed in both non-smoking and smoking women, their frequency was increased in women with a current smoking history (P=0.0177 for α2,3-sialic acid-positive cysts; P=0.0802 for α2,6-sialic acid-positive cysts) (Fig. 4). Therefore, active smoking increases the incidence of sialic acid-containing ciliated cysts in the Fallopian tube epithelium.

Discussion

Sialyltransferase expression was previously reported for some human female reproductive tissues, including the placenta and ovary. Here, we extend their expression profile, revealing that six members of the ST3GAL family and two members of the ST6GAL family are expressed in the human Fallopian tube during the menstrual cycle. Each enzyme targets glycoproteins, oligosaccharides, and glycolipids as acceptor substrates, with some selectivity among the different members (Ishii et al, 1998; Kitagawa et al, 1994). The ubiquitously expressed ST6GAL1 (Krzewinski-Recchi et al, 2003), for example, transfers sialic acid to the galactose β1,3/4 *N*-acetylglucosamine (Galβ1,3/4GlcNAc) structure on glycoproteins and oligosaccharides (Harduin-Lepers et al, 2001). We observed changes in *ST6GAL1* transcript abundance in the Fallopian tube during the secretory phase, implying that the glycan structure also changes during the menstrual cycle. Indeed, more α2,6-sialylation may be present in the Fallopian tube epithelium during the secretory phase; whether or not this functions to inhibit ectopic implantation of the blastocyst is not clear.

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Addition of sialic acid to an epithelial surface will augment its negative charge. Blastocysts possess a predominately negatively charged surface (Nilsson et al, 1975), which could be repelled by the enriched 2,6-sialylation in the Fallopian tube epithelium. Conversely, sialylation is reduced in the decidua during early pregnancy (Jones et al, 2010), possibly making the endometrium more favorable for blastocyst implantation. Indeed, reduced expression of *ST6GAL1*, *ST3GAL3*, *ST3GAL4*, and *ST3GAL5*, within the Fallopian tube samples from women with an ectopic pregnancy strongly supports a model whereby less sialylation may foster implantation in the Fallopian tube.

Galectin-1 and -3 are β-galactoside-binding animal lectins with a high affinity for lactosamine residues (Galβ1,3/4GlcNAc). Galectins are expressed at implantation sites, suggesting their involvement in the establishment of pregnancy (Nio-Kobayashi et al, 2015). Galectin-1 expression increased in cases of ectopic implantation, and trophoblast-derived molecules enhanced epithelial galectin-1 expression (Nio-Kobayashi et al, 2015). The sugar-binding affinity for galectin-1 is blocked by α2,6-sialylation on terminal galactose, which is the product of ST6GAL1 activity. Increased *ST6GAL1* expression during the secretory phase could participate in blocking galectin-1 binding, thereby inhibiting implantation in the Fallopian tube. We cannot determine if reduced *ST6GAL1* transcript abundance in the Fallopian tube from women with ectopic implantation was a cause or effect of the ectopic implantation; however, less *ST6GAL1* was measured in the Fallopian tube of women with a history of smoking, which is a risk factor for tubal ectopic pregnancy.

Despite altered expression of some β-galactoside sialyltransferases, especially *ST6GAL1*, during the menstrual phase and ectopic pregnancy, the presence and localization of both α2,3- and

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α2,6-sialic acids did not change appreciably. Histochemical analysis is not quantitative, so we cannot comment on exact sialylation levels in the Fallopian tube using this technique. Further experiments utilizing mass spectrometry would be required to determine if whole glycan structures change during the menstrual phase and with or without ectopic implantation. Such results would also inform the contribution of other sialyltransferases that use non-galactose acceptors, given that current reagents limit our ability to discriminate all sialic acid acceptor sugar structures.

Revealing the function of galectins and glycoconjugates during blastocyst implantation and the pathogenesis of ectopic pregnancy requires identfication of the glycoconjugates, especially those carrying α2,6-sialyltion. Mucin 1 (MUC1) is recognized by galectins (Jeschke et al. 2009; Mori et al. 2015), making it a candidate glycoprotein that may associate with galectins in the Fallopian tube and uterus. MUC1 is a highly glycosylated protein whose decreased surface expression allows blastocysts to implant at the endometrium in normal pregnancy as well as on the Fallopian tube epithelium in tubal ectopic pregnancy (Aplin et al, 2001; Savaris et al, 2008; AI-Azemi et al, 2009). Cigarette smoking alters MUC1 glycosylation, which is involved in the epithelial-to-mesenchymal transition in the airway (Zhang et al. 2014). Therefore, investigation of the change in MUC1glycan structure in the endometrium of non-fertile women versus the Fallopian tube epithelium from women with ectopic implantation would be a great interest.

We also observed the formation of sialic acid-containing ciliated cysts in the epithelium of the Fallopian tubes. Ciliated cysts are present in ciliated epithelium of oviducts from various animals, including humans (Hagiwara, 2000), and are considered markers of abnormal ciliogenesis. Inhibiting

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the migration of duplicated centrioles, using cytochalasin D, also induces these intracellular cysts in cultured oviducts of quails (Boisvieux-Ulrich et al, 1990). In this study, all histological sections examined contained ciliated cysts, but their number was significantly increased in the Fallopian tube from women who were confirmed smokers compared to confirmed non-smokers. These data suggest that the increased number of ciliated cysts reflects impaired ciliogenesis in the Fallopian tube epithelium of women who smoked. Cigarette smoking is a major risk factor for tubal ectopic implantation (Shaw et al, 2010), and we previously showed that luminal epithelial cells of the Fallopian tube of women who smoke have altered rates of cell turnover and a higher potential to lose cilia (Horne et al. 2014). Rresults from this study provide additional evidence that smoking changes the structure of the Fallopian tube epithelial cells.

The transcript abundance of two sialyltransferases – a reduction in *ST6GAL1*, as discussed above, and an increase in *ST3GAL5* (ganglioside GM3 synthase) – was significantly altered in the Fallopian tube from women who smoked. Ganglioside GM3 synthesis is involved in ciliary development; indeed, *St3gal5* knockout mice have impaired hearing because of a deformity in hair cells in the organ of Corti (Yoshikawa et al, 2009), implying that ganglioside GM3 is essential for the normal ciliary structure in this organ. Whether or not increased *ST3GAL5* can be linked to the abnormal ciliary structures in smokers, perhaps as a consequence of abnormal ciliogenesis or potentially a cause of inclusion cysts, is not clear. Further experiments are required to understand the precise function of sialylation and its role in ciliogenesis, ciliary function, and ectopic implantation. Nevertheless, cilia dysfunction might slow the transit of a blastocyst, as well as cause functional

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changes in the Fallopian tube that promote ectopic implantation.

In conclusion, the present study demonstrates that decreased sialylation in the Fallopian tube correlates with the pathogenesis of tubal ectopic pregnancy. Decreased α2,6-sialylation may enhance the binding of galectin-1, thereby increasing receptivity for the blastocyst in Fallopian tube epithelium. Smoking is associated with reduced α2,6-sialylation-involved gene expression and with increased ciliated cysts, which are linked with abnormal ciliary function. We therefore conjecture that smoking alters tubal transport (ciliary action) and enhances the potential for ectopic implantation, which are both marked by changes in sialic acids.

Materials & Methods

Human Fallopian tube collection

Ethical approval for this study was obtained from the Lothian Research Ethics Committee (LREC 10/S1102/40), with informed written consent from all of the participating women. Serum samples and the Fallopian tube biopsies from the ampulla region of the Fallopian tube were collected at the time of hysterectomy for benign gynecological conditions or during surgical management of tubal ectopic pregnancy.

Women were 18−45 years of age. The menstrual phase of each non-pregnant patient at the time of hysterectomy was determined by histologic examination and staging of an endometrial biopsy taken with the Fallopian tube and by measurement of serum estradiol and progesterone levels, as described previously (Duncan et al, 2011). Fallopian tube samples from ectopic pregnancies were

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confirmed to be free of trophoblast contamination, as described previously (Nio-Kobayashi et al, 2015). Patients were identified as smokers or non-smokers based on patient history combined with assessment of serum cotinine concentration, which is greater than 100 ng/mL in women with a smoking history but negligible in non-smokers (Horne et al, 2014). .

Biopsies of the human Fallopian tubes were divided into equal portions and either immersed in RNAlater (Ambion, TX, USA) at 4˚C overnight and then flash frozen at −80°C for RNA extraction, or fixed in 10% neutral-buffered formalin overnight at 4˚C followed by storage in 70% ethanol and subsequent embedding in paraffin for histological staining.

Quantitative reverse-transcription PCR

The Fallopian tube tissues used for a quantitative gene expression analysis were examined from confirmed non-smoking women (n=14 total; proliferative phase, n=5; mid-secretory phase, n=9); from women with a confirmed history of smoking (n=6 total; proliferative phase, n=3; mid-secretory phase, n=3); or from non-smoking women with ectopic pregnancy (n=13). Total RNA was extracted from frozen human Fallopian tubes using an RNeasy Mini Kit (Qiagen Ltd., Crawley, UK,), according to the manufacturer's protocol. RNA (200 ng) was used to prepare cDNA using TaqMan Reverse Transcription reagents (Applied Biosystems, Foster City, CA, USA).

Primers (Table 3) were pre-validated by standard PCR, and then by generating standard curves using quantitative PCR. Each reaction buffer contained 5.0 µL 2×PowerSYBR® Green PCR Master Mix (Applied Biosystems), 0.5 µL primer pair (5 µM), 3.5 µL of nuclease free H_2O, and 1.0 µL

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cDNA; each reaction was performed in duplicate. The PCR cycling program consisted of a denaturing step (95˚C for 10 min), annealing and extension steps (95˚C for 15 sec and 60˚C for 1 min, repeated for 40 cycles), and a dissociation step (95˚C, 60˚C, and 95˚C for 15 sec each) using a 7900 Sequence Detection System (Applied Biosystems). The relative abundance of each target, normalized to the housekeeping gene (*G6PDH*), previously validated using geNorm analysis (Primerdesign Ltd, Southampton, UK), was quantified using the ΔCt method. After testing for normality, all statistical analyses were performed by unpaired *t*-tests using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA); $P<0.05$ was regarded as significant. Values in the tables represent the mean ± standard error of expression of the target genes relative to *G6PDH*.

Lectinhistochemistry

Fixed human Fallopian tube tissues from confirmed non-smokers (n=7 total; proliferative phase, n=2; mid-secretory phase, n=5); from confirmed smokers (n=7 total; proliferative phase, n=2; mid-secretory phase, n=5); and with ectopic pregnancy (n=8) were used for histological analysis. Sections (5 μm thick) were de-waxed and washed in phosphate-buffered saline (PBS). Sections were first incubated with a Avidin/Biotin blocking solution (Vector Laboratories Inc., Burlingame, CA), and then blocked for 60 min at room temperature using Carbo-free blocking solution (Vector Laboratories Inc.). Sections were then incubated in PBS at 4˚C overnight with 1:250 dilutions of biotinylated lectins from *Maackia amurensis* (MAM) or *Sambucus sieboldiana* (SNA) (Seikagaku corporation, Tokyo, Japan), which specifically recognizes α2,3- or α2,6-sialic acids, respectively, on

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glycoconjugates (these products are catalysed by sialyltransferases including ST3GAL and ST6GAL). The silaic acid-containing sites were visualized by reacting for 60 min with reagents of a Vectastain ABC Elite kit, followed by a 5-min development with an ImmPACT^{TM} DAB Peroxidase Substrate Kit (Vector Laboratories Inc.). The sections were counterstained with haemotoxylin, and observed under a light microscope (BX51; Olympus corporation, Tokyo, Japan).

Images of all tissue sections stained with MAM or SNA were used to quantify of the number of MAM- or SNA-positive ciliated cysts per section. The epithelium area was measured using Image J (http://imagej.nih.gov/ij/), and used to calculate the number of cysts per 1 mm^2 epithelium. The difference in the number of ciliated cysts during the mid-secretory phases (n=5 from non-smokers and smokers) between the samples was analysed by unpaired *t*-tests using GraphPad Prism 6 software. $P<0.05$ was regarded as significant. Values in the graphs represent the mean ± standard error.

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Figure legends

Figure 1. Changes in sialyltransferases mRNA abundance in human Fallopian tubes from women with or without a smoking history.

The expression of (**A**) *ST3GAL5* or (**B**) *ST6GAL1* in the proliferative phase or mid-secretory phase Fallopian tube from confirmed smokers and confirmed non-smokers. Means ± standard errors are plotted. The number of individuals assess is shown per group. *P* values for the significantly different pair-wise comparisons are indicated.

Figure 2. Localization of α2,3- and α2,6-sialic acids in human Fallopian tube. Lectin histochemistry using plant lectins that specifically recognize α2,3-sialic acids (MAM) or α2,6-sialic acids (SNA), which are catalyzed by sialyltransferases including ST3GAL and ST6GAL families. Both α2,3- and α2,6-sialic acids are found in the stroma (**A**, **B**); at the apical surface of the epithelium (**C**, **D**); and on cilia of ciliated cells as well as on the apical surface of non-ciliated secretory cells (**E**, **F**). Arrows indicate the non-ciliated cell surface. Note the small vesicles in the cytoplasm of both ciliated cells and secretory cells exhibiting SNA-reactivity (F). Scale bars, 200 μm (A-B); 50 μm (C-D); or 10 μm (E-F).

Figure 3. Formation of sialic acid-containing ciliated cysts in the Fallopian tube epithelium.

MAM- and SNA-positive round structures were found in various depths of the epithelium (**A**-**B,** arrows), which were identified as ciliated cysts resulting from abnormal ciliogenesis. Condensed

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MAM reactivity is found along the concave bottom of the ciliary tuft (**C,** arrows) as well as in jug-like structures with contact to the luminal surface of the epithelium (**D**, arrow). In other cells, MAM-positive cysts are detached from the luminal surface (**E**, arrow) or embedded in the basal region of the epithelium (**F**, arrow). Asterisks show the nuclei of the ciliated cells with cysts. Scale bars, 20 µm (A-B) or 10 µm (C-F).

Figure 4. The effect of smoking on the number of ciliated cysts in the Fallopian tube epithelium. The number of both MAM- and SNA-positive ciliated cysts in the mid-secretory phase Fallopian tube of confirmed smokers and confirmed non-smokers (n=5). Means ± standard errors are plotted. *P* values for the significantly different pair-wise comparisons are indicated.

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Table 1. Relative mRNA expression of sialyltransferases in the Fallopian tube from non-pregnant women without smoking history and women with ectopic implantation

Gene	Non-pregnancy		Comparison of phases in non-pregnant women	Ectopic pregnancy	Comparison of non-pregnant v. ectopic pregnancy women (mid-secretory phase)
	Proliferative (n=5)	Mid-secretory (n=9)		(n=13)	
ST3GAL1	2.68 ± 0.59	4.54 ± 0.64	n.s.	5.35 ± 0.83	n.s.
ST3GAL2	3.02 ± 0.83	2.80 ± 0.21	n.s.	2.60 ± 0.41	n.s.
ST3GAL3	0.42 ± 0.08	0.47 ± 0.06	n.s.	0.26 ± 0.03	P=0.0029
ST3GAL4	1.00 ± 0.21	1.10 ± 0.08	n.s.	1.19 ± 0.12	n.s.
ST3GAL5	0.62 ± 0.17	0.78 ± 0.06	n.s.	0.55 ± 0.06	P=0.0089
ST3GAL6	1.63 ± 0.61	1.71 ±0.29	n.s.	0.74 ± 0.13	P=0.001
ST6GAL1	2.34 ± 0.43	8.28 ± 0.74	P<0.0001	3.63 ± 0.67	P<0.0001
ST6GAL2	0.20 ± 0.08	0.16 ± 0.03	n.s.	0.27 ± 0.06	n.s.

Values are shown as mean ± standard error. Statistical comparisons were analyzed by Student's *t*-test.

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Table 2. Changes in sialyltransferases mRNA abundance in the Fallopian tube of women with or without smoking history

Gene	**Non-smokers** (n=14)	**Smokers** (n=6)	**Comparison of non-smokers and smokers**
	Proliferative stage (n=5)	Proliferative stage (n=3)	
	Mid-secretory stage (n=9)	Mid-secretory stage (n=3)	
ST3GAL1	3.87 ± 0.52	3.36 ± 0.31	n.s.
ST3GAL2	2.88 ± 1.03	3.15 ± 0.40	n.s.
ST3GAL3	0.45 ± 0.05	0.48 ± 0.07	n.s.
ST3GAL4	1.07 ± 0.09	1.24 ± 0.12	n.s.
ST3GAL5	0.72 ± 0.07	1.30 ± 0.34	P=0.0169
ST3GAL6	1.68 ± 0.27	2.02 ± 1.85	n.s.
ST6GAL1	6.16 ± 0.98	4.80 ± 1.84	n.s.
ST6GAL2	0.17 ± 0.03	0.19 ± 0.03	n.s.

Values are shown as mean ± standard error. Statistical comparisons were analyzed by Student's *t*-test.

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Table 3. Primers used for quantitative reverse-transcription PCR

Genes	Accession no.	Forward	Reverse	Product size (bp)
G6PDH	NM_000402	5'-CGGAAACGGTCGTACACTTC	5'-CCGACTGATGGAAGGCATC	155
ST3GAL1	NM_003033	5'-GGCCTGAGATAGACAGTCACG	5'-CTCACCACCCACTCCAAGTC	187
ST3GAL2	NM_006927	5'-CAGCCACTTTGACGGTAACA	5'-AGCACCTCATTGGTGTTGTG	126
ST3GAL3	NM_174963	5'-AGTCGCAGGATTTGGCTATG	5'-CTGGGTCTGAAGACTCCTGTG	259
ST3GAL4	NM_006278	5'-TTGGCTCTGGTCCTGGTC	5'-GATCCTCACTCCCCTTGGTC	238
ST3GAL5	NM_003896	5'-TGTGGACCCTGACCATGTAA	5'-CTGGCAAGAGTTCCAAGAGG	242
ST3GAL6	NM_006100	5'-TGGAATTGTTGATGGGTGAC	5'-ACCAGCTAGGTGAACTTCGTG	242
ST6GAL1	NM_173216	5'-CTGCCCAAGGAGAGCATTAG	5'-TGTTGGAAGTTGGCTGTGG	158
ST6GAL2	NM_032528	5'-CGTGGTTATGAGAAAGATGTTGG	5'-GGCTGATTTGGGTTTCTCTG	230

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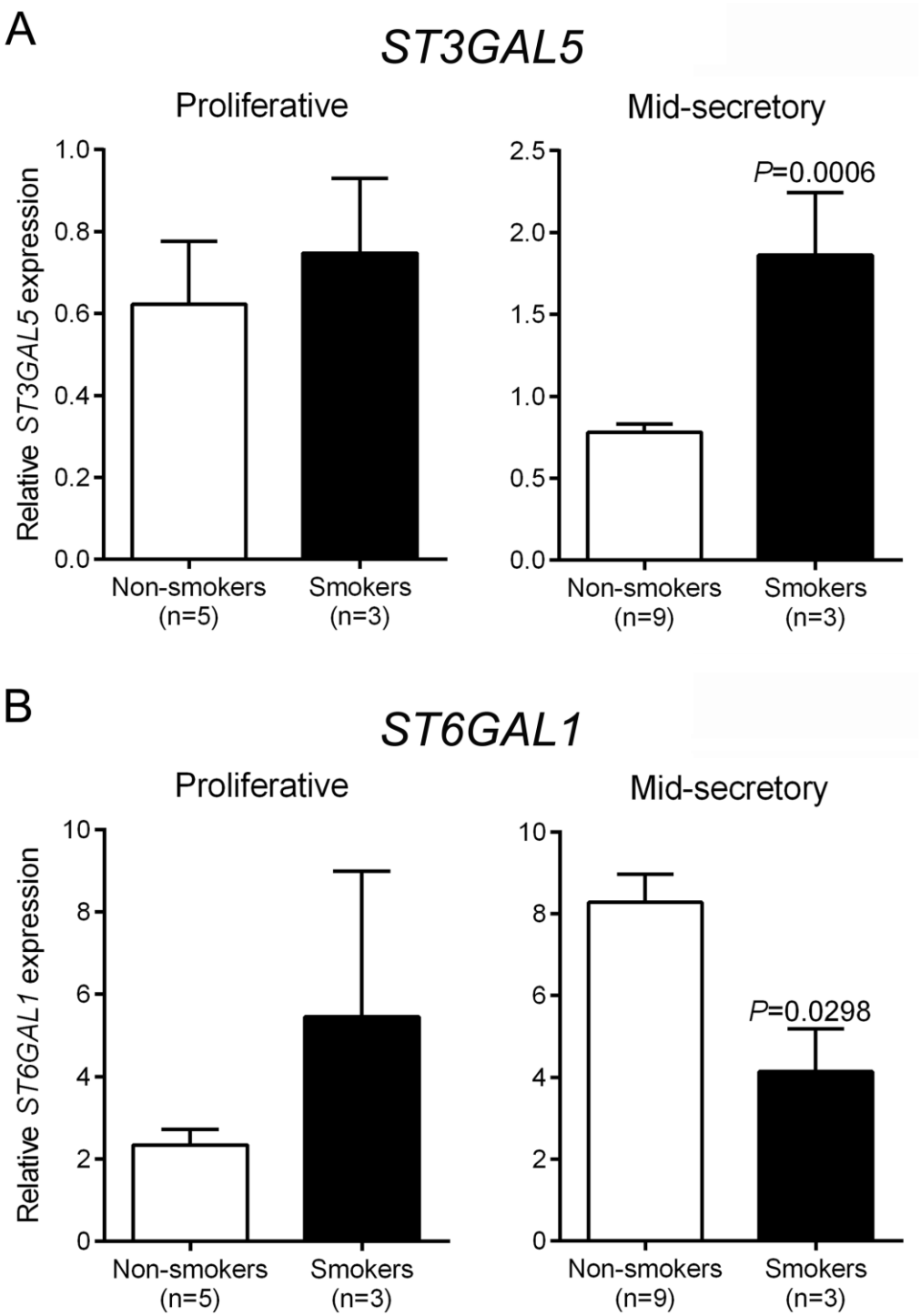

Figure 1

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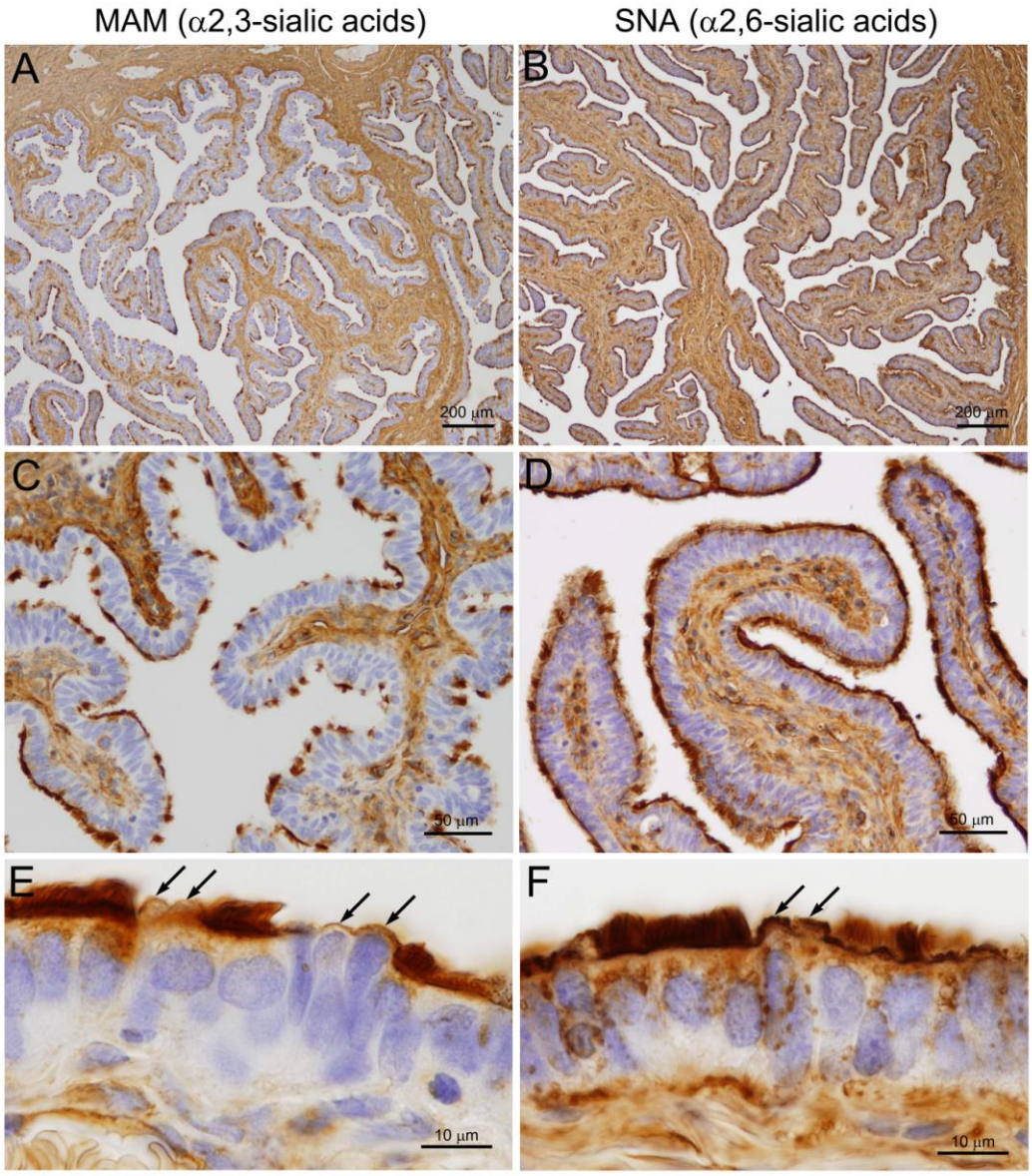

Figure 2

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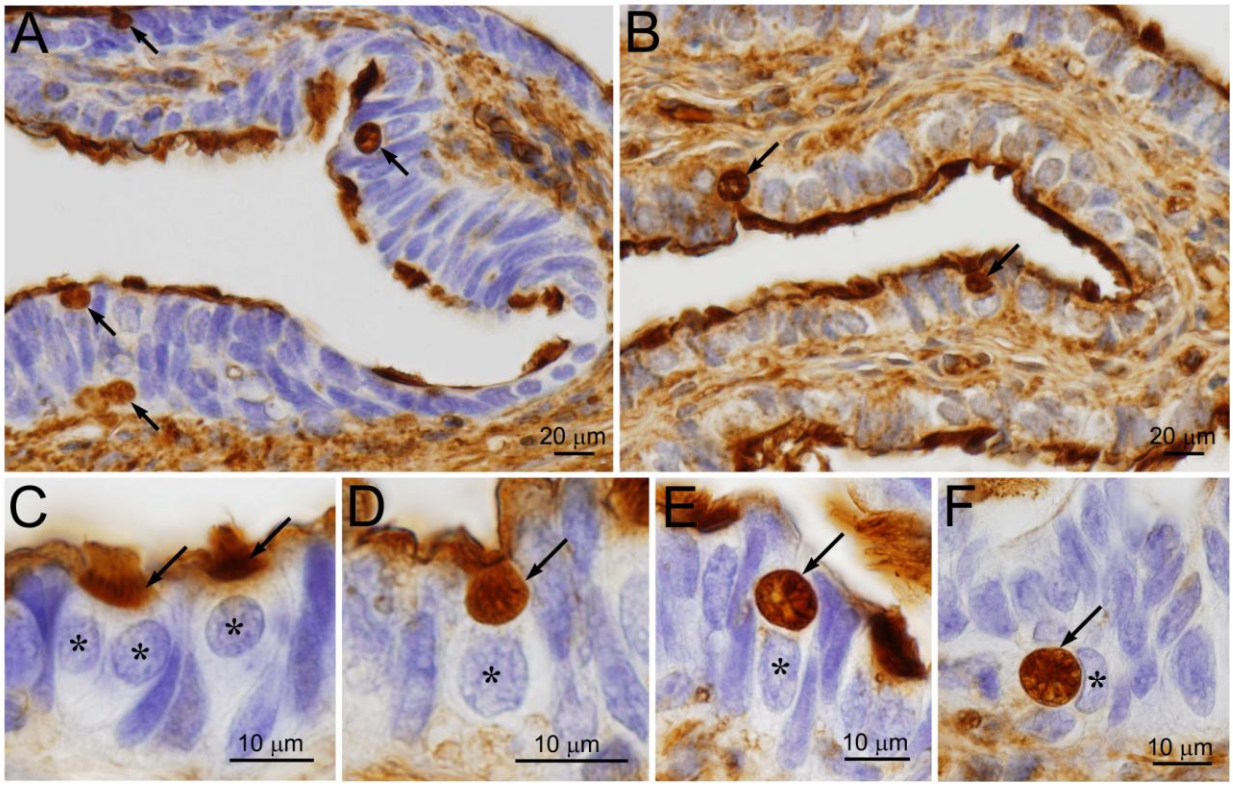

Figure 3

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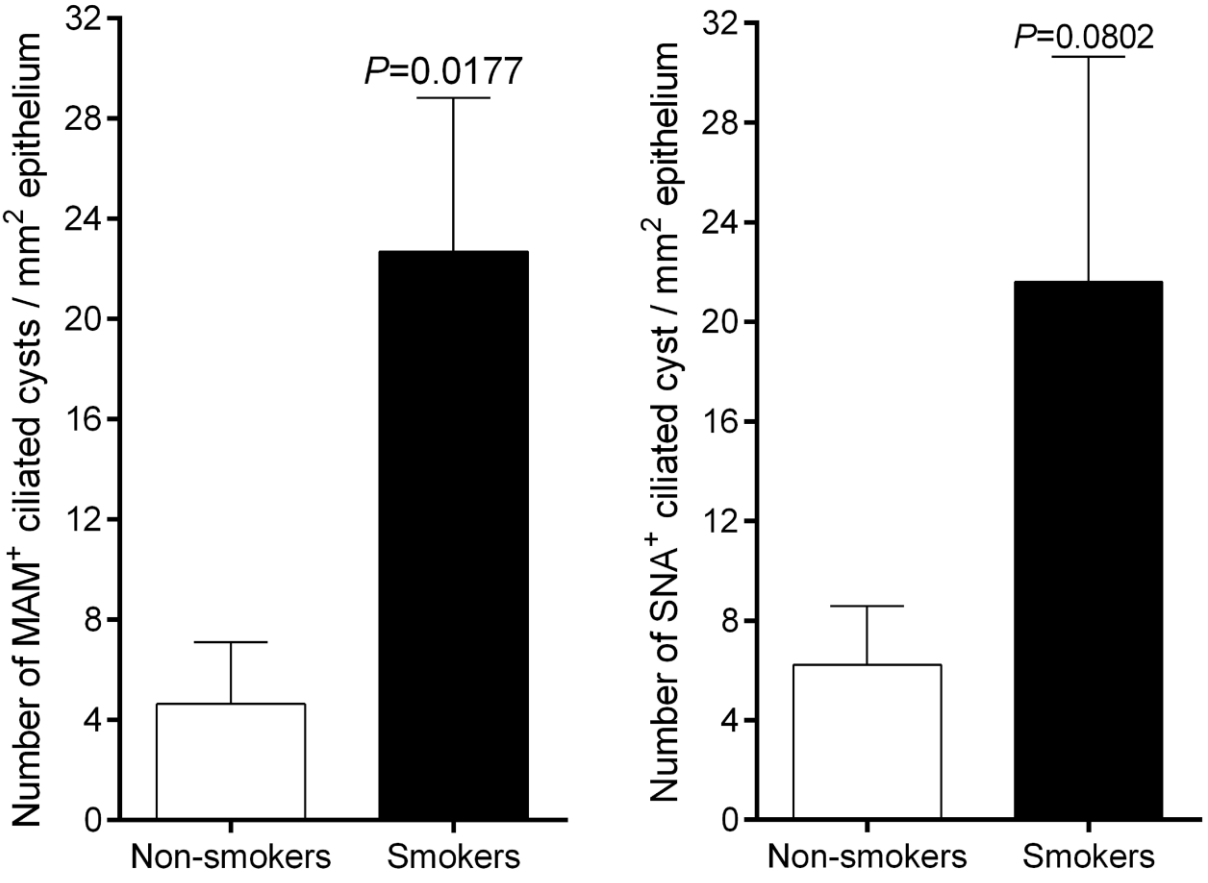

Figure 4

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