Localization and variable expression of $\text{G}_\alpha_{i2}$ in human endometrium and fallopian tubes

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Running title: $\text{G}_\alpha_{i2}$ in human reproductive tissues
Abstract:

BACKGROUND: Heterotrimeric G proteins take part in membrane-mediated cell-signalling and have a role in e.g. hormonal regulation. This study clarifies the expression and localization of the G protein subunit \( \alpha_{i2} \) in the human endometrium and fallopian tube and changes in \( \alpha_{i2} \) expression in human endometrium during the menstrual cycle. METHODS: The expression of \( \alpha_{i2} \) was identified by PCR, and localization confirmed by immunostaining. Cyclic changes in \( \alpha_{i2} \) expression during the menstrual cycle were evaluated by quantitative real time PCR. RESULTS: We found \( \alpha_{i2} \) to be expressed in human endometrium, fallopian tube tissue and fallopian tube primary epithelial cells. Our studies revealed enriched localization of \( \alpha_{i2} \) in human fallopian tube cilia and in endometrial glands. We showed that \( \alpha_{i2} \) expression in human endometrium changes significantly during the menstrual cycle. CONCLUSIONS: \( \alpha_{i2} \) is specifically localized in oviductal cilia of rat and human and is likely to have a cilia-specific role in reproduction. Significantly variable expression of \( \alpha_{i2} \) during the menstrual cycle suggests it might be under hormonal regulation in the female reproductive tract \textit{in vivo}. 
Introduction

Among the cell-surface receptors, G protein-coupled receptors are the most widespread and
diverse, playing an essential regulatory role in cell growth, hormonal regulation, sensory
perception and neuronal activity (Hepler and Gilman, 1992). In reproduction, G protein-
coupled receptors have a neuroendocrine regulatory role in gonadotropin-releasing hormone
(GnRH) -induced secretion of luteinising hormone (LH) and follicle-stimulating hormone
(FSH) from the anterior pituitary gland (Chi et al., 1993; Tsutsumi et al., 1992). In gonads, G
protein-coupled receptors mediate gonadotropin signalling (Loosfelt et al., 1989; McFarland
et al., 1989; Minegishi et al., 1991; Minegishi et al., 1990; Sprengel et al., 1990), thus
regulating the synthesis and secretion of sex hormones.

G protein-coupled receptors communicate via heterotrimeric G proteins, which are recognized
as crucial elements in various types of membrane-mediated cell-signalling. Heterotrimeric G
proteins consist of α-, β- and γ-subunits. According to the α-subunits, G proteins are divided
into four classes (Gs, Gi, Gq and G12) (Hepler and Gilman, 1992). Proteins of the Gi family are
the most diverse and interact with a wide variety of G protein-coupled receptors. For example,
they take part in hormonal regulation via interaction with GnRH (Hawes et al., 1993;
Krsmanovic et al., 2003; Krsmanovic et al., 2001; Stanislaus et al., 1998), FSH (Arey et al.,
1997) and LH receptors (Herrlich et al., 1996). Moreover, Gi family proteins play a role in the
signal transduction of rapid, nongenomic actions of estrogen (Benten et al., 2001) and
progesterone (Karteris et al., 2006; Zhu et al., 2003).

The dual balance between Gi and Gs signalling in the regulation of adenylyl cyclase has been
well established. Proteins of Gi-family can inhibit adenylyl cyclase (AC) and thus decrease
intracellular cAMP concentration (Bokoch et al., 1984; Katada et al., 1984). Via this pathway, G\textsubscript{i}-family protein G\textsubscript{a\textsubscript{i2}} has been shown to take part in adrenergic signalling, controlling myometrium relaxation in the rat during pregnancy (Mhaouty et al., 1995). In the human myometrium, the levels of G\textsubscript{a\textsubscript{i2}} have been shown to decrease during pregnancy, suggesting that the consequent, altered balance between G\textsubscript{a\textsubscript{i2}} and G\textsubscript{s} could be responsible for maintaining the relaxation of uterus during pregnancy (Europe-Finner et al., 1993). Although the role of G\textsubscript{a\textsubscript{i2}} in myometrium has been thoroughly studied, the presence or the role of G\textsubscript{a\textsubscript{i2}} elsewhere in the human reproductive tract remains unclear.

Immunohistochemical studies in the rat have shown that G\textsubscript{a\textsubscript{i2}} is specifically localized in tissues having motile cilia with a characteristic 9+2 ultrastructure. Such a specific localization in rat oviductal, tracheal and brain ependymal cilia (Shinohara et al., 1998) implies that G\textsubscript{a\textsubscript{i2}} may well serve a physiological function distinct from those of the other G\textsubscript{a} subunits. It is probable that G\textsubscript{a\textsubscript{i2}} might play a cilia-specific physiological role. Interestingly, proteomic analysis has revealed G\textsubscript{a\textsubscript{i2}} as a resident axonemal protein of the human bronchial cilia (Ostrowski et al., 2002). To date, however, there are no reports providing evidence of the localization of G\textsubscript{a\textsubscript{i2}} in any other human ciliated tissues, such as fallopian tubes. In this study, we identify the presence and localization of G\textsubscript{a\textsubscript{i2}} in tissues which are primarily in contact with gametes, and provide environment for fertilization, early development of the embryo as well as implantation, i.e., the human fallopian tube and endometrium. We have also evaluated the potential changes in G\textsubscript{a\textsubscript{i2}} expression in human endometrium during the menstrual cycle to reveal any potential hormonal regulation of this G protein subunit in humans.
Materials and methods

Endometrial tissue collection and preparation for immunohistochemistry

The current study was approved by the Local Ethics Committee and informed written consent was obtained prior to the collection of tissue samples. For immunohistochemical investigations, tissue samples were obtained from 6 fertile women, and for genomic studies, endometrial biopsies were obtained from 21 fertile women. All the women taking part in the investigation had regular cycles, showed no evidence of any pathological uterine disorder, and had not used oral contraception or an intrauterine device during the previous three months.

Biopsies were obtained in the operating theatre between 2 and 29 days after the last menstrual period (LMP). The mean age of the women taking part in the study was 35 (range 24-40) years, and each had had at least one previous successful pregnancy.

Endometrial biopsies for immunohistochemistry were immediately snap-frozen and stored in liquid nitrogen until processed. Cryosections were cut at 5 µm and stored at -70°C until use. For genomic studies, endometrial biopsies were immediately placed in RNAlater (Ambion, Huntingdon, U.K.), followed by immersion in liquid nitrogen until processed.

Fallopian tube tissue collection and preparation for immunohistochemistry

Human fallopian tube tissues were collected from 9 patients undergoing total abdominal hysterectomy for benign gynaecological conditions. The mean age of the women taking part in the study was 42 (range 33-56) years.

Fallopian tube tissue samples for immunohistochemistry were immediately fixed in 10% formalin overnight and embedded in paraffin. Paraffin sections were cut at 5 µm. For genomic
studies, fallopian tube tissue samples were immediately placed in RNAlater (Ambion), and stored for 24 hours at 4°C followed by immersion and storage in liquid nitrogen until processed.

**Cell culture**

Fallopian tube tissue samples for primary epithelial cell cultures were obtained as follows: fallopian tubes were placed in Hank’s solution immediately after collection, cut open longitudinally and incubated 1 h with 0.25 % collagenase (at 37°C, 95% O₂, 5% CO₂). The cells were scraped gently using a sterile blade, washed with red blood cell lysing buffer (Sigma-Aldrich) and then 2-3 times with culture media (DMEM-F12). The cells were plated into 75 ml flasks. Fallopian tube primary epithelial cells were cultured at +37°C in DMEM (F12) culture media (Invitrogen, Paisley, UK) supplemented with 1% penicillin and streptomycin (Sigma-Aldrich), 10% fetal calf serum (Invitrogen) and L-glutamine (Invitrogen) in 5% CO₂ atmosphere.

**RNA isolation and cDNA synthesis**

Tissues were removed from RNAlater and homogenised in 3 ml of TRIreagent (Sigma-Aldrich) using an Ultra-Turrax homogenizer for 2 min. Total RNA from the tissues and pelleted cells stored in TRIreagent was extracted following standard protocol supplied by the manufacturer. Total RNA was treated with Dnase I (DNA-free™, Ambion) to remove genomic DNA contamination from the samples. First strand cDNA synthesis was performed using oligo dT primers (Metabion, Martinsried, Germany) and reverse transcription by SuperScript II (200 U/µl, Invitrogen, Paisley, UK). Negative controls were prepared without the enzyme (non-reverse transcribed controls, RT controls).
PCR

PCR was performed with the constructed cDNAs, Platinum Blue PCR Super Mix (Invitrogen) and primers from Metabion. We used the following primer pairs: β-actin forward 5'-TGA CCC AGA TCA TGT TTG AGA CC-3' and β-actin reverse 5'-GGA GGA GCA ATG ATC TTG ATC TTC-3', Gα2 forward 5'-CTT GTC TGA GAT GCT GGT AAT GG-3' and Gα2 reverse 5'-CTC CCT GTA AAC ATT TGG ACT TG-3'. The amplification was run for 35 cycles under the following conditions: 95° 30 sec, 58° or 65° 30 sec, 72° 30 sec. Amplified sequences were 643 and 212 base pairs for Gα2 and β-actin respectively. Annealing temperatures of 58° (β-actin) and 65° (Gα2) were used. All experiments included RT controls as well as negative controls (no cDNA). PCR products were separated on 1.2 % agarose gel.

Quantitative real time PCR

Quantitative real time PCR was performed with the constructed cDNAs and the same primers that were used in PCR reactions. SYBR Green Jump Start (Sigma-Aldrich) master mix (containing 10µl SYBR Green, 7µl Water, 1µl of each primer and 1µl cDNA) was added to each well of PCR plate and amplification was performed under the following conditions: 50 cycles (95° 30 sec, 58° or 65° 30 sec, 72° 30 sec). All experiments included RT controls and negative controls (no cDNA).

Results were analyzed using iCycler (Biorad laboratories Ltd, Hemel Hempstead, UK). To compare relative quantities of Gα2 expression during the menstrual cycle, endometrial biopsies were divided into three groups; menstrual (LMP + 1-4; n = 3; LMP +1, +4 and +4), proliferative (LMP + 5-14; n = 9; early proliferative LMP +5, +5 and +7, mid-proliferative LMP +8, +9 and +10, late proliferative LMP +11, +12 and +13) and secretory (LMP + 15-29; n = 9; early secretory LMP +16, +16 and +17, mid-secretory LMP +20, +21 and +22, late
secretory LMP +26, +28 and +29). Relative $G_{\alpha_{i2}}$ expression quantities were compared between these groups. The threshold cycle values were normalised against threshold value of human $\beta$-actin. The results were expressed as mean ± S.E.M. Statistical analysis was performed by using one-way ANOVA with Tukey’s multiple comparison test. $p < 0.05$ was considered significant.

### Immunohistochemistry

Cryosections of endometrium were thawed by immersion (15 min at 20 °C) into fixative containing 4 % paraformaldehyde (Sigma-Aldrich, Poole, UK) in 0.1 M PBS, pH 7.4. The slides were then washed with PBS (2x5 min), and further fixed by immersion in -20°C methanol (4 min) followed immediately by treatment with -20°C acetone (2 min). After 2x5 min washes with PBS, endogenous peroxidase activity was blocked by 5% H$_2$O$_2$ (in distilled water) treatment (5 min). The slides were then washed with deionized water (2x5 min) and PBS (2x5 min). After this, the protocol follows the same blocking and staining protocol as described for paraffin sections.

Fallopian tube paraffin sections were firstly dewaxed in xylene, rehydrated through a series of ethanols and finally washed with PBS. Endogenous peroxidase activity was quenched by a 20 min incubation with 3% H$_2$O$_2$ (v/v) in methanol. Antigen retrieval was performed by microwave irradiation in 10mM citrate buffer, pH 6.0 (12 min). The slides were allowed to cool in the buffer and then washed with PBS (2x3 min).

Vectastain Elite ABC Kit (Vector Laboratories, Peterborough, UK) was used according to the manufacturers instructions for both cryosections and paraffin sections, with the following modifications. Slides were blocked in blocking buffer containing 250 μl avidin D / ml (1 h
Mouse anti-\( \alpha \)-2 monoclonal antibody, MAB3077 (Chemicon International, Temecula, CA) was diluted into Dako antibody diluent (Dako UK Ltd, Cambridgeshire, UK) containing 250 µl biotin / ml, and incubated overnight at 4 ºC (cryosections 1:1000, paraffin sections 1:500). Primary antibody was omitted in negative controls. The slides were washed with PBS (5 min), and incubated with secondary antibody (1:200 Biotinylated anti-mouse (Vector Laboratories)) for 30 min at 20 ºC. The slides were washed as before and incubated for 30 min with Vectastain ABC reagent (Vector Laboratories). After washing, binding was visualized by incubation with substrate DAB or DAB-Ni for 8 min (Vector Laboratories). The slides were rinsed with tap water (5 min) and PBS (3 min) and counterstained by using 10% haematoxylin (10 min). Following thorough rinse in tap water, slides were dehydrated through a series of ethanols, cleared in xylene and coverslipped with DePex mounting medium (VWR International, Lutterworth, UK).

The endometrial biopsy specimens were timed according to LMP and morphology and divided into three groups, menstrual, proliferative or secretory. The slides were imaged using a x40 objective on an Olympus CKX41 microscope. Digital images were captured with a Nikon Coolpix 5400 camera and identically edited in Adobe Photoshop (Adobe Systems, Mountain View, CA).
Results

**PCR reveals the expression of G\(\alpha_{i2}\) gene in human reproductive tissues.**

We used human fallopian tube tissue and human endometrial biopsies to study the expression of \(G\alpha_{i2}\) by PCR. Our data revealed that \(G\alpha_{i2}\) is expressed in human fallopian tube and human endometrium (Figure 1 A, B). Our studies also confirmed that \(G\alpha_{i2}\) is expressed in primary cultures of fallopian tube epithelial cells (Figure 1 C). Control experiments with non-reverse transcribed RNA of each sample confirmed that there was no contamination of human DNA in the samples.

**Immunohistochemistry shows specific localization of G\(\alpha_{i2}\) protein in fallopian tube cilia and enrichment in endometrial glands.**

Immunostaining on human fallopian tube paraffin sections showed specific localization of \(G\alpha_{i2}\) protein in fallopian tube epithelial cells and the cilia (Figure 2 C). Positive staining was also seen in the cytoplasm of epithelial cells, surrounding the nuclei. In endometrial tissue, \(G\alpha_{i2}\) staining was enriched in endometrial glands, but was present also in stroma (Figure 2 A, B).

**Quantitative real time PCR shows alterations in G\(\alpha_{i2}\) gene expression during the menstrual cycle.**

We carried out quantitative real time PCR experiment on endometrial biopsies spanning the menstrual cycle (Figure 3). Based on the phase of the menstrual cycle of each patient, the biopsies were designated in three groups, namely menstrual (LMP + 1-4), proliferative (LMP + 5-14) and secretory (LMP + 15-29).
Our results demonstrated that endometrial expression of $G_{\alpha_{i2}}$ gene changed during the cycle. The expression reached its peak in secretory phase. The expression of $G_{\alpha_{i2}}$ gene in secretory phase was significantly higher ($p < 0.05$) compared to that of the other phases.
Discussion

The present study demonstrates the existence and localization of $\alpha_{i2}$ in human endometrium and fallopian tube. Our data establishes the specific localization of $\alpha_{i2}$ in the fallopian tube epithelial cells, particularly in the cilia of fallopian tube epithelial cells. In human endometrium, we have demonstrated that localization of $\alpha_{i2}$ is enriched in endometrial glands. We have also shown that $\alpha_{i2}$ expression in human endometrium changes significantly during the menstrual cycle with maximum expression in the secretory phase, providing evidence that expression of this $G_i$ subunit might be under hormonal regulation in the female reproductive tract \textit{in vivo}.

The presence of G protein subunit $\alpha_{i2}$ in rat myometrial membranes was first reported by Milligan \textit{et al.} (1989) and the finding was later supported by a study suggesting differential regulation of $\alpha_{i2}$ and $\alpha_{i3}$ in rat myometrium during gestation (Tanfin \textit{et al.}, 1991). In human myometrium, the levels of G protein subunits $\alpha_{i1}$, $\alpha_{i3}$, $\alpha_q$ and $\alpha_{11}$ have been shown to remain constant in pregnant and non-pregnant women, while levels of $\alpha_{i2}$ decrease during pregnancy. The simultaneous, substantial increase in myometrial $G_s$ suggested that the balance between $\alpha_{i2}$ and $G_s$ might be essential in regulating relaxation of the uterus during pregnancy (Europe-Finner \textit{et al.}, 1993). Besides this, $G_i$ family proteins have been suggested to be functionally linked to $\alpha_2$ adrenergic signalling in human myometrium during pregnancy (Breuiller \textit{et al.}, 1990). Later studies in the rat have confirmed the involvement of $\alpha_{i2}$ and $\alpha_{i3}$ in $\alpha_2/\beta_2$ adrenergic signalling in the maintenance of uterus relaxation during rat pregnancy (Mhaouty \textit{et al.}, 1995).
Unlike the thoroughly studied myometrium, the presence and role of $G\alpha_{i2}$ in other regions of the reproductive tract has remained largely obscure. Although the presence of $G_\text{i}$ family proteins have been described in human endometrium during artificial cycles of hormone replacement therapy, those studies rely solely on data from immunoblotting, using an antibody unable to discriminate between the closely related $G\alpha_{i1}$ and $G\alpha_{i2}$ (Bernardini et al., 1995, 1999). Therefore, prior to our study, cyclical changes in $G\alpha_{i2}$ expression have not been reported in humans. Quantitative PCR showed that $G\alpha_{i2}$ expression in human endometrium in vivo significantly increased towards secretory phase of the menstrual cycle. This suggested that sex hormones, like oestrogen or progesterone, might regulate the expression of this $G_\text{i}$ subunit in human endometrium. Furthermore, immunostaining clearly demonstrated the main localization of $G\alpha_{i2}$ in endometrial glands and partially in endometrial stroma.

It is likely that $G\alpha_{i2}$ is hormonally regulated in the human endometrium. Earlier studies on rat myometrium have shown that estradiol administration during rat pregnancy increases the levels of both $G\alpha_{i2}$ and $G\alpha_{i2}$ mRNA, while progesterone has no effect on $G\alpha_{i2}$ expression. Instead, progesterone was reported to cause a decrease in $G\alpha_q$ subunit expression (Cohen-Tannoudji et al., 1995). Other studies in pregnant rat myometrium have suggested a regulatory role for progesterone in control of $\beta_2$ receptors (Maltier et al., 1989) and $G_s$ proteins (Elwardy-Merezak et al., 1994), as well as in upregulation $\beta_2$ receptor expression (Vivat et al., 1992). Apart from the studies by Bernardini et al. (1995; 1999) the potential role for sex hormones in regulation of $G$ proteins in the human has remained largely unexplored.

In the present study, we have reported for the first time the localization of $G\alpha_{i2}$ in fallopian tube epithelial cilia. In fallopian tubes, ciliary beat is essential for gamete transport in association with the tubal secretory flow and muscle contractility. Furthermore, fallopian
tubes have been proposed to act as sperm reservoirs, where the ciliated epithelial cells interact with sperm (Baillie et al., 1997; Pacey et al., 1995a; Pacey et al., 1995b; Reeve et al., 2003). Fallopian tube epithelial cells have also been demonstrated to preserve the viability of sperm (Kervancioglu et al., 1994; Kervancioglu et al., 2000; Murray and Smith, 1997). Given the fact that $\alpha_{i2}$ is specifically localized in rat tissue motile cilia with a characteristic 9+2 ultrastructure, namely in rat oviductal, tracheal and brain ependymal cilia (Shinohara et al., 1998), it seems evident that this $G_i$ subunit might have a cilia-specific physiological role. Apart from proteomic analysis providing evidence of $\alpha_{i2}$ as a resident axonemal protein of the human bronchial cilia (Ostrowski et al., 2002), there are no reports describing $\alpha_{i2}$ in any other human ciliated tissue. In addition to positive immunostaining of fallopian tube cilia, we reported here positive immunostaining surrounding the nuclei. This presumably represents pre-stage $\alpha_{i2}$ which is still in synthesis, or alternatively, $\alpha_{i2}$ which is ready for transport into cilia by intraflagellar transport mechanisms. This intracellular machinery is vital for assembly and maintenance of the cilia, as it transports essential particles, such as proteins synthesised in the cytoplasm of cell, into the cilia, and returns the turnover products to the cytoplasm of cell (Rosenbaum and Witman, 2002). Studies with $\alpha_{i2}$-knockout mice have established a crucial regulatory role for the $\alpha_{i2}$ subunit in immunological processes (Dalwadi et al., 2003; Fan et al., 2005; Han et al., 2005; Jiang et al., 1997; Rudolph et al., 1995; Rudolph et al., 1995; Zhang et al., 2005). $\alpha_{i2}$ has been revealed to control regulation of T-cell proliferation (Zhang et al., 2005) and B cell development (Dalwadi et al., 2003). Furthermore, $\alpha_{i2}$ has been suggested to mediate chemokine signalling (Han et al., 2005). However, reports of $\alpha_{i2}$-knockout studies have not provided any information on potential involvement of this $G_i$ subunit in modulation of mice fertility. Interestingly, a recent study on $\alpha_{i2}$-knockout mice showed $\alpha_{i2}$ to differentially
regulate inflammatory mediator production in response to microbial stimuli and proposed a TLR-signalling regulating, anti-inflammatory role for $G_{\alpha_i2}$ by an yet unknown mechanism (Fan et al., 2005). Regarding the potential link between TLR-signalling and $G_{\alpha_i2}$ in female reproductive tract, it is noteworthy that our previous studies showing the localization pattern of several TLRs (Fazeli et al., 2005) showed a similar pattern of localisation compared to that we now report for $G_{\alpha_i2}$. Future studies should be directed towards understanding whether $G_{\alpha_i2}$ might share signalling pathways with TLRs, and potentially have a TLR-signalling regulating role in human reproductive tract.

In conclusion, our studies reveal the presence of $G_{\alpha_i2}$ in human endometrium and fallopian tube epithelium, especially the cilia of fallopian tube epithelial cells. To the best of our knowledge, this is the first report of the localization of $G_{\alpha_i2}$ in ciliated reproductive tissue in the human. We also report here, for the first time, the alterations in $G_{\alpha_i2}$ expression during human menstrual cycle. Our data implies this $G_i$ family subunit might be under hormonal regulation in the female reproductive tract in vivo. Further studies are required to clarify the physiological role of $G_{\alpha_i2}$ in the female reproductive tract.
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References


Figure legends

Figure 1. PCR showed $G_{\alpha_i2}$ expression in fallopian tube tissue (A), human endometrium tissue (B) and fallopian tube primary epithelial cells (C). PCR products were separated on 1.2% agarose gel. 1: $\beta$-actin (643 base pairs), 2: $\beta$ actin RT control, 3: $G_{\alpha_i2}$ (212 base pairs), 4: $G_{\alpha_i2}$ RT control, $M_W$: molecular weight (base pairs).

Figure 2. Immunostaining showing localization of $G_{\alpha_i2}$ in human endometrial cryosections and fallopian tube paraffin embedded sections. $G_{\alpha_i2}$ is enriched in endometrial glands, proliferative phase (A), secretory phase (B). Immunostaining of human fallopian tube paraffin embedded sections (C) indicated specific localization of $G_{\alpha_i2}$ in fallopian tube epithelial cells and the cilia. $G_{\alpha_i2}$ (brown). Negative control slides were incubated with diluent only. All the slides were counterstained with haematoxylin (blue). Scale bar: 100 $\mu$m (A, B), 40 $\mu$m (C).

Figure 3. Quantitative real time PCR uncovered variable expression of $G_{\alpha_i2}$ gene in endometrium during the menstrual cycle. Endometrial biopsies were designated in three groups according to menstrual history of the patient (menstrual n=3, proliferative and secretory n=9). The figure illustrates mean ± SEM of normalised $G_{\alpha_i2}$ gene expression. * Secretory phase was significantly different from the other phases, $p < 0.05$; One-way ANOVA with Tukey’s multiple comparison test.
**Figure 1.** PCR showed $\alpha_{i2}$ expression in fallopian tube tissue (A), human endometrium tissue (B), immortalized fallopian tube epithelial cell line (OE-E6/E7) (C) and fallopian tube primary epithelial cells (D). PCR products were separated on 1.2 % agarose gel. 1: $\beta$-actin (643 base pairs), 2: $\beta$ actin RT control, 3: $\alpha_{i2}$ (212 base pairs), 4: $\alpha_{i2}$ RT control, $M_W$: molecular weight (base pairs).

**Figure 2.** Immunostaining shows localization of $\alpha_{i2}$ in human endometrial cryosections and fallopian tube paraffin embedded sections. $\alpha_{i2}$ is enriched in endometrial glands, proliferative phase (A), secretory phase (B). Immunostaining of human fallopian tube paraffin embedded sections (C) indicated specific localization of $\alpha_{i2}$ in epithelial cells and the cilia. $\alpha_{i2}$ (brown): Chemicon MAB3077 primary antibody was used with dilutions of 1:1000 for endometrial cryosections and 1:500 for paraffin embedded fallopian tube sections. DAB or DAB-Ni was used as a chromogen (endometrial cryosections and paraffin embedded fallopian tube sections, respectively). Negative control slides were incubated with diluent only. All the slides were counterstained with haematoxylin (blue). Scale bar: 100 µm.

**Figure 3.** Western blot analysis confirmed the presence of $\alpha_{i2}$ in immortalized fallopian tube epithelial cell line (OE-E6/E7). A: G protein standard, (2 µl / lane) Bovine brain immunoblot standard, Calbiochem. B: Homogenate of fallopian tube epithelial cells, (60 µg / lane).

**Figure 4.** Quantitative real time PCR uncovered variable expression of $\alpha_{i2}$ in endometrium during the menstrual cycle. Endometrial biopsies were designated in three groups according to menstrual history of the patient (menstrual n=3, proliferative and secretory n=9). The figure illustrates mean ± SEM of normalised $\alpha_{i2}$ gene expression. * Secretory phase was
significantly different from the other phases, \( p < 0.05 \); One-way ANOVA with Tukey’s multiple comparison test.
Figures

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