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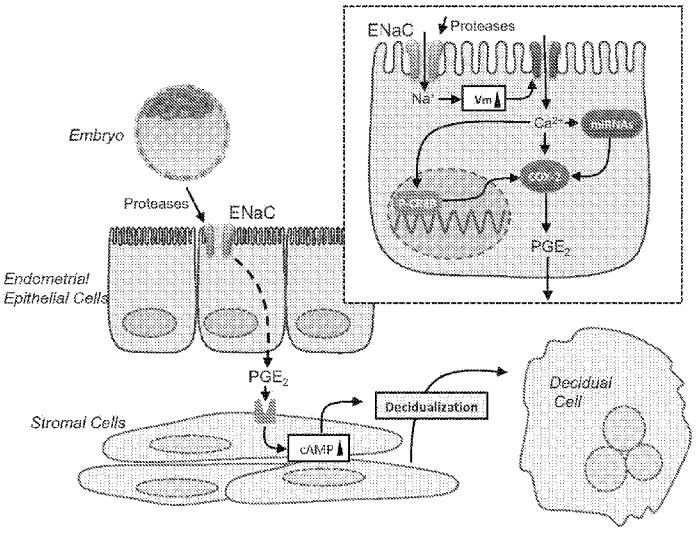

FIG. 5

(57) Abstract: This invention is based on the discovery that activation of the endometrial epithelial sodium channel (ENaC) triggers PGE2 release and production, which is required for embryo implantation. This invention provides a system for developing pharmaceuticals that inhibit ENaC activity or decrease ENaC expression (for example, using small molecule drugs or siRNA). These pharmaceuticals can be used for birth control or contragestion, or to treat inflammatory conditions mediated by PGE2. This invention also provides a system for developing pharmaceuticals that activate endometrial ENaC or increasing its expression. These pharmaceuticals can be used for promoting embryo implantation.

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CONTRAGESTION AND TREATING INFLAMMATION BY MODULATING SODIUM CHANNEL ACTIVITY IN THE EPITHELIUM

RELATED APPLICATION

[0001] This International Application claims the priority benefit of U.S. patent application 61/623,520, filed April 12, 2012. The priority application is hereby incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates generally to the fields of fertility, birth control, pregnancy, *in vitro* fertilization, parturition, and inflammatory disease. More specifically, it relates to modulation of epithelial sodium channel (ENaC) activity as a means for achieving contragestion, maintaining pregnancy, or treating an inflammatory condition.

BACKGROUND

[0003] The world population is projected to continue its grow until at least 2050, with the population reaching 9 billion in 2040. Some predictions put the population in 2050 as high as 11 billion. Almost all growth will take place in the less developed regions, where today's 5.3 billion population of underdeveloped countries is expected to increase to 7.8 billion in 2050. Overpopulation strains resources that are fundamental to human existence, including fresh water, food, housing, energy, and the environment.

[0004] Accordingly, there is a need for new technologies that makes birth control more effective and more readily accessible throughout the world.

SUMMARY OF THE INVENTION

[0005] The inventors have discovered that activation of the epithelial sodium channel (ENaC) triggers PGE2 release and production. ENaC activity in the endometrium is required for embryo implantation. This invention provides a system for developing pharmaceuticals that inhibit ENaC activity or decrease ENaC expression (for example, using small molecule drugs or siRNA). These pharmaceuticals can be used for birth control or contragestion, or to treat inflammatory conditions mediated by PGE2. This invention also provides a system for developing pharmaceuticals that activate endometrial ENaC or increase its expression. These pharmaceuticals can be used for promoting embryo implantation.

[0006] One embodiment of this invention is a method of identifying a pharmaceutical product as an agent for birth control or contragestion. The method typically comprises the steps of obtaining a compound that inhibits transport of sodium ion through an endometrial epithelium sodium channel; administering said compound to a test subject when an embryo is present in the uterus of the subject following fertilization; and then determining whether the compound affects implantation of said embryo in the uterus of the subject. This leads to a method for producing a pharmaceutical composition for birth control, the method comprising obtaining a compound that inhibits ENaC activity or expression, and formulating an effective amount of the compound with a pharmaceutically compatible excipient for administration to the uterus of a human subject. Compounds that inhibit transport of sodium ion through ENaC can be identified by combining the compound with cells or a culture systems that express functional ENaC, and measuring the effect of the compound on the flux of sodium ion. Other assays for the identification, screening, and validation of potential therapeutic agents are provided in the description that follows.

[0007] Another embodiment of the invention is a pharmaceutical composition formulated and labeled for use in birth control in a human subject, comprising an effective amount of a compound that inhibits an endometrial epithelium sodium channel. The compound can be a small molecule drug exemplified by but not limited to amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET). Alternatively, it can be antisense RNA, interfering RNA, or siRNA that inhibits expression of said endometrial epithelium sodium channel; or it can be an antibody that specifically binds and thereby blocks said endometrial epithelium sodium channel.

[0008] The invention also provides a method of birth control, contragestion, inducing labor, by inhibiting COX-2 activity, or by inhibiting production of prostaglandins such as PGE2 in the uterus of a subject. The method comprises administering to the subject an effective dose of an inhibitor of an endometrial epithelium sodium channel. The inhibitor may be administered into the uterus of the subject: either by way of injection, or by way of topical application.

[0009] Another embodiment of the invention is a method for improving the probability of implantation in a subject of an *in vitro* fertilized (IVF) embryo. The method comprises increasing the expression or activity of an epithelium sodium channel (ENaC) in the endometrium in the subject: for example, by administering into the uterus of a subject an effective amount of a serine protease.

[0010] A further embodiment of the invention is a method of identifying a pharmaceutical product as an agent for treating an inflammatory condition. The method generally comprises the steps of obtaining a compound that inhibits transport of sodium ion through an epithelium sodium channel, as already described. The compound is then administered to a test subject who has an inflammatory condition; and the ability of the compound to reduce inflammation is determined. This leads to a method for producing a product for treating an inflammatory condition in which an effective amount of an ENaC inhibitor is formulated with a pharmaceutically compatible excipient

for administration to a human subject: either systemically, or locally at or around the site of inflammation.

[0011] Another embodiment of the invention is a pharmaceutical composition formulated and labeled for use in treating an inflammatory condition in a human subject, comprising an effective amount of a compound that inhibits an epithelium sodium channel. The compound can be a small molecule drug exemplified by but not limited to amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET). Alternatively, it can be antisense RNA, interfering RNA, or siRNA that inhibits expression of said epithelium sodium channel; or it can be an antibody that specifically binds and thereby blocks the epithelium sodium channel.

[0012] The invention also provides method of treating an inflammatory condition in a subject, comprising administering to the subject an effective dose of an inhibitor of an epithelium sodium channel. The method comprises administering to the subject an effective dose of an inhibitor of an epithelium sodium channel at or near the site of the inflammation.

[0013] Other embodiments of the invention will be apparent from the description that follows.

DESCRIPTION OF THE DRAWINGS

[0014] **Figure 1** illustrates ENaC-mediated signal transduction events and PGE2 release from mouse endometrial epithelial cells. **Figure 1(A)**, Trypsin-induced membrane depolarization assessed by voltage-sensitive fluorometric measurement. **Figure 1(B)**, Changes in intracellular Ca^{2+} levels in endometrial epithelial cells (EECs) in response to trypsin in the presence of amiloride or nifedipin. **Figure 1(C)**, Trypsin-induced PGE2 release from 3-day culture of EEC. **Figure 1(D)**, Quantitative PCR analysis of COX-2 mRNA levels in EECs. **Figure 1(E)**, Western blot analysis of protein levels of phosphorylated form of CREB (P-CREB). **Figure 1(F)**, Fluorescence labeling of P-CREB in EECs with DAPI labeled nucleus. **Figure 1(G)**, qPCR analysis of miR199a and miR101 levels in EECs.

[0015] **Figure 2** shows involvement of ENaC in trypsin-induced decidualization in endometrial epithelial/stromal cell co-culture. **Figure 2(A)**, are bright-field photographs of cocultured stromal cells before and after treatment with trypsin. **Figure 2(B)**, shows trypsin-induced intracellular cAMP increase in the co-culture, which could be inhibited by amiloride.

[0016] **Figure 3** shows the requirement of ENaC activity for embryo implantation in vivo. **Figure 3(A)**, Effect of intrauterine injection of amiloride, EIPA or aprotinin on implantation rate in mice. **Figure 3(B)**, Western blotting data showing the effectiveness of in vivo knockdown of ENaCα (the alpha chain of ENaC) by intrauterine injection of siRNA. **Figure 3(C)**, Effect of ENaCα knockdown on implantation rate in mice with a photograph showing implantation sites (arrowed) in the control uterine horn (siRNANC) as compared with treatment using siRNAENaCα. **Figure 3(D)**, Hematoxylin and eosin stains samples showing the effect of ENaCα knockdown on

decidualization in mice on day 5, with less decidual cells observed as compared with the control. **Figure 3(E)**, Effect of ENaCα knockdown on uterine miRNA (micro RNA) levels, measured by qPCR, on day 4 and day 7. **Figure 3(F)**, Western blot analysis showing the effect of ENaCα knockdown on the uterine expression of COX-2.

[0017] **Figure 4** provides a Western blot analysis that compares endometrial expression of ENaCα between women undergoing IVF treatment with successful and failed pregnancy.

[0018] **Figure 5** is a sketch depicting the role of ENaC in initiating decidualization.

[0019] **Figure 6(A)** shows L-type Ca2+ channel expression in mouse endometrial epithelial cells (EECs). **Figure 6(B)** is a patch-clamp recording of Ca2+ channel activities (n=7) before (left) and after (right) addition of nifidepine, a small-molecule calcium channel blocking drug.

[0020] **Figure 7(A)** shows the effect of ENaCα knockdown on decidualization in mice. The number of cells with morphology of decidual cells was significantly reduced in the siRNA-treated uteri, compared with the control. This illustrates the involvement of ENaC activity in embryo implantation. **Figure 7(B)** shows that interference with ENaC expression with siRNA substantially reduced the expression of implantation markers HoxA10, IgF2 and LIF.

[0021] **Figure 8** provides clinical features of a group of patients undergoing IVF (in vitro fertilization).

[0022] **Figure 9** is a compilation of endometrial expression of ENaC amongst women in this study. The endometrial samples from women with failed pregnancy after IVF-ET had significantly lower ENaCα subunit expression.

[0023] **Figures 10(A)** and **10(B)** are Western blots showing phosphorylation of CREB by COX-2. **Figure 10(A)** shows results of treating cells with the ENaC activator trypsin. **Figure 10(B)** shows results of actiatinv cells by mechanical stimulation. Inhibition of ENaC expression or function can have an anti-inflammatory effect when administered either lcoally or systemically.

DETAILED DESCRIPTION

[0024] The amiloride-sensitive epithelial sodium channel(ENaC), coded by SCNN1 genes within the degenerin/ENaC superfamily, is localized in the apical membrane of a wide variety of epithelia including endometrial epithelium. It is known for its essential role in salt and water homeostasis. (Amiloride-sensitive sodium channel subunit beta [Homo sapiens]: Accession: P51168.2 GI: 8928561; Amiloride-sensitive sodium channel subunit alpha: Accession: P37088.1 GI: 585966; Amiloride-sensitive sodium channel subunit gamma: Accession: P51170.4 GI: 108885072; Amiloride-sensitive sodium channel subunit delta: Accession: P51172.2 GI: 116242784; SEQ. ID NOs:7 to 12).

[0025] The data presented in this disclosure show that activation of an epithelial ion channel can trigger signaling cascade leading to increased release and production of PGE2. It has been

discovered that interfering with ENaC in the uterus (either by channel inhibitors or specific siRNA) can suppress COX-2 and PG release, resulting in implantation failure in mice (Example 3). Furthermore, abnormal ENaC expression is found in IVF patients with implantation failure (Example 4).

[0026] **Figure 5** provides a working model for how ENaC mediates decidualization. ENaC activation by serine proteases from the embryo causes epithelial cell membrane depolarization that triggers calcium influx leading to: (1) release of PGE2; (2) phosphorylation of CREB that may up-regulate the expression of COX-2; and (3) alteration in COX-2-targeting micro-RNA. The endometrial epithelial released PGE2 in turn activates cAMP-related pathways in stromal cells, leading to stromal decidualization. This model is provided to assist the reader in understanding possible mechanisms that underlie aspects of the invention, without conveying a limitation on how the invention is practiced.

[0027] Thus, inhibiting ENaC activity and/or reducing its expression in the uterus is a modality for contragestion. Conversely, increasing ENaC activity and/or enhancing its expression in the uterus is a modality for improving the probability of implantation in a subject of an *in vitro* fertilized (IVF) embryo. Given the wide distribution of the ion channel and the importance of COX-2 and prostaglandins in inflammation, inhibiting ENaC activity and/or reducing its expression is also a new modality for treating inflammatory conditions throughout the body.

Molecular biology of the Amiloride Sensitive Sodium Channel

[0028] The amiloride-sensitive epithelial sodium channel (ENaC) is a constitutively active ion-channel located in various tissues of the body. ENaC is located in the apical membrane of polarized epithelial cells particularly in the kidney (primarily in the collecting tubules), the lung and the colon. ENaC consists of three different subunits: specifically, α, β, and γ, or alternatively δ, β, and γ. Each of the subunits consists of two transmembrane helices and an extracellular loop. ENaC is involved in the transepithelial Na+-ion transport, which it accomplishes together with the Na+/K+-ATPase.

[0029] Kellenberger et al. have provided a review of the epithelial sodium channel/degenerin family of ion channels and their structure. Physiol Rev. 2002; 82(3):735-67. Chan et al. have investigated the distribution and regulation of ENaC in the murine female reproductive tract. J Membr Biol. 2002;185(2):165-76. Yang et al., have investigated differential expression and localization of ENaC in mouse endometrium during preimplantation. Cell Biol Int 28, 433-9 (2004). Quadri et al. have reviewed ENaCs as therapeutic targets. Am J Physiol Cell Physiol. 2012 (e-pub). For an overview, the reader may also refer to the text AMILORIDE-SENSITIVE SODIUM CHANNELS: PHYSIOLOGY AND FUNCTIONAL DIVERSITY, D.J. BENOS, 1999.

Inhibiting ENaC activity or decreasing ENaC expression as a method of birth control

[0030] As shown in Examples 1 and 2, activation of ENaC in mouse endometrial epithelial cells by an embryo-released serine protease triggers Ca^{2+} influx that leads to PGE2 release, phosphorylation of transcription factor CREB, upregulation of cyclooxygenase 2 (COX-2), the enzyme important for prostaglandin (PG) production and implantation, and alteration of two COX-2-targeting the micro-RNAs: specifically, miR-199a and miR-101. As shown in Example 3, blocking or knocking down expression of uterine ENaC results in implantation failure accompanied with altered levels of uterine COX-2, miR-199a and miR-101.

[0031] **Figure 3(A)** shows that intrauterine injection with amiloride (an ENaC inhibitor) in an animal model on day 3 of pregnancy can induce dose-dependent reduction in the number of implanted embryos. **Figure 3(B)** shows significantly reduced levels of ENaC α-chain in the uteri injected with siRNAENaCα compared with control **Figure 3(C)** shows a reduction in implantation rate that occurs from knocking down expression of the ENaC alpha chain using siRNA.

[0032] Accordingly, the invention provides a method of birth control, contragestion, inducing labor, or inhibiting PGE2 production in the uterus by administering to the subject an effective dose of an inhibitor of an endometrial epithelium sodium channel.

Activating ENaC or increasing its expression as a method of promoting embryo implantation

[0033] As shown in Example 4 and **Figures 4**, **8**, and **9**, ENaC expression in the uterus prior to implanting an *in vitro* fertilized (IVF) embryo is significantly lower in women with implantation failure as compared with those with successful pregnancy. These results demonstrate that endometrial ENaC has a role in regulating PGE2 production and release required for embryo implantation. Defects in ENaC function may be a cause of miscarriage and low successful rate in IVF.

[0034] Thus, the invention provides a method for improving the probability of implantation in a subject of an embryo or blastocyst that is transplanted into the uterus: including but not limited to an *in vitro* fertilized (IVF) embryo. The method comprises increasing the expression or activity of an endometrial epithelium sodium channel (ENaC) in the subject: for example, by administering into the uterus of a subject an effective amount of a serine protease.

Inhibiting ENaC activity or decreasing ENaC expression as a method of treating inflammation

[0035] ENaC is traditionally viewed to play a primarily role in electrolyte and fluid reabsorption, hyperactivity of which can lead to dehydration of lumens, particularly the airways, giving rise to bacterial infection, thus inflammation, such as in the case of cystic fibrosis. Many currently available anti-inflammation drugs target COX-2 directly.

[0036] **Figures 10(A) and 10(B)** show that ENaC plays a role in regulating COX-2 and prostaglandin release outside the uterus. This provides a basis for inhibiting ENaC activity or decreasing its expression as a method of treating inflammatory conditions of different kinds.

[0037] Thus, the invention also provides method of treating an inflammatory condition. An effective dose of an inhibitor of an epithelium sodium channel at or near the site of the inflammation, thereby inhibiting COX-2 activity and the production of prostaglandins like PGE2. The method comprises administering to the subject an effective dose of an inhibitor of an epithelium sodium channel at or near the site of the inflammation.

Agents for inhibiting ENaC activity or decreasing ENaC expression

[0038] In order to inhibit ENaC activity or decrease its expression according to this invention, different options are available. An overview is provided in the reference ION CHANNELS AND THEIR INHIBITORS, S.P. Gupta, 2011.

Small molecule drugs

[0039] Small molecule drugs are known and have been developed that interfere with ENaC function. Vallet et al., Nature 389, 607-10 (1997), described the use of amiloride to inhibit ENaC. Kashlan et al. , J. Biol. Chem. 285:35216-35223, 2010, provides Ac-LHPHLQRL-amide as a potential ENaC inhibitor.

[0040] Other small-molecule ENaC inhibitors include arachidonic acid and its metabolites 11,12-epoxyeicosatrienoic acid (11,12-EET), 8,9-EET, and 14,15-EET. Sun et al., J. Am. Assoc. Nephrol. 21:1667-1677, 2010; Pavlov et al., Am J Physiol Renal Physiol. 2011 Sep;301(3):F672-81. Other ENaC inhibitors include BAY39-9437 (CT Poll et al., Bayer Pharmaceuticals, Am J Physiol Lung Cell Mol Physiol 281:L16-L23, 2001), as well as those disclosed in international patent applications WO 07/071,400 and WO 07/071,396.

[0041] To identify small molecule drugs suitable for inhibiting ENaC according to this invention, compounds can be screened using a cell line that expresses ENaC constitutively. To measure ligand binding, isotopically labeled compounds are combined with the cells, and the label associated with the cells is determined after washing. To measure the effect on ENaC activity, test compounds are combined with the cells, and transport or flux of sodium ion across the membrane of the cells is monitored. A test compound can be identified as having an ability to inhibit ENaC activity if the cells treated with the compound show less transport of sodium ion than the same cells before or without treatment, or other cells that have been treated with a compound known not to modulate ENaC function. To measure the effect of a compound on ENaC protein expression, the compound is combined with the cells, and then the level of ENaC protein on the cells is determined by immunoassay or Western blot, in comparison with untreated control cells.

Antisense technology

[0042] Another approach is to inhibit ENaC expression by way of interfering RNA and other antisense technology. See generally the texts THERAPEUTIC OLIGONUCLEOTIDES: ANTISENSE, RNAI, TRIPLE-HELIX, GENE REPAIR, ENHANCER DECOYS, CPG, AND DNA CHIPS; Yoon S. Cho-Chung, A.M. Gewirtz et al, 2003; and ANTISENSE THERAPEUTICS, M.I. Phillips, 2010. siRNA and other types of antisense RNA can be created against any of the chains of human ENaC or the animal model being used (SEQ ID NOs:3, 5, 7, 9, and 11).

[0043] Small interfering RNA (siRNA) reduces ENaC expression by way of the RNA interference pathway. siRNAs typically have a short (~21-nucleotide) double-strand RNA with 2-nucleotide 3' overhangs on either end. Standard RNA chemistry can be adapted to improve specificity, stability and/or biocompatibility. Exemplary is Stealth RNAi™ technology, developed at *Sequitur Inc.*, and available commercially from *Life Technologies*, Grand Island, NY, U.S.A. By way of illustration, siRNA for inhibiting expression of the ENaC alpha chain is AAA GCA AAC UGC CAG UAC AUG C (SEQ ID NO:1) and GCA UGA UGU ACU GGC AGU UUG CUU U (SEQ ID NO:2). To confirm the effect of siRNA on the expression of one or more subunits of ENaC, the siRNA is combined with cells that constitutively express ENaC, and then the level of the corresponding ENaC subunit protein on the cells is determined by immunoassay or Western blot, in comparison with untreated control cells.

[0044] For further reading on the preparation and use of siRNA, the reader is referred to RNA INTERFERENCE: FROM BIOLOGY TO CLINICAL APPLICATIONS, W.P. Min et al., 2010; RNA INTERFERENCE: APPLICATION TO DRUG DISCOVERY AND CHALLENGES TO PHARMACEUTICAL DEVELOPMENT, P.H. Johnson et al., 2011.

Antibodies

[0045] Antibodies specific for an endometrial ENaC can be prepared according to standard protocols of immunization, harvesting antibody-expressing cells, immortalizing the harvested cells (for example, using Epstein Barr virus), and selecting clones that produce antibody having the desired specificity. The immunogen can be any of the ENaC chains or a fragment thereof produced by recombinant expression, or a synthesized peptide comprising a portion of an ENaC chain amino acid sequence (SEQ ID NOs:4, 6, 8, 10 or 12). Specific single-chain variable regions can be produced by contacting a library of immunocompetent cells or viral particles with the target antigen, and growing out positively selected clones. Marks et al., New Eng. J. Med. 335:730, 1996, and patent publications WO 94/13804, WO 92/01047, and WO 90/02809.

[0046] Specific antibody can be also be custom ordered from commercial services such as *Pacific Immunology*, Ramona, CA, U.S.A.; *Precision Antibody*, Columbia, MD, U.S.A., and *AbMax Biotechnology Co.*, Ltd., Beijing, China. Binding of antibody to cell-surface ENaC can be

determined in cells constitutively expressing ENaC by combining the antibody with the cells, and then determining antibody bound by immunohistochemistry for immunoglobulin. The ability of antibody to affect the presentation of ENaC on the cell surface (for example, by promoting endocytosis) can be determined by combining the antibody with the cells, and then determining the level of ENaC expression by immunohistochemistry for ENaC antigen. The ability of antibody to block ENaC function can be determined by combining the antibody with the cells, and measuring the effect on the flux of sodium ion.

[0047] For further reading on the preparation and use of therapeutic antibodies and their equivalents, the reader is referred to THERAPEUTIC MONOCLONAL ANTIBODIES: FROM BENCH TO CLINIC, Z. An, 2009; THERAPEUTIC ANTIBODIES: METHODS AND PROTOCOLS, A.S. Dimitrov, 2009; and RECOMBINANT ANTIBODIES FOR IMMUNOTHERAPY, M. Little, 2009.

Agents for activating ENaC or increasing its expression

[0048] As described in more detail below, selected agents that activate ENaC or increase its expression can be used in the preparation of pharmaceuticals for promoting embryo implantation into the uterine wall, thereby improving the rate of pregnancy.

[0049] V, Vallet et al., Nature 389, 607-10 (1997), describes an epithelial serine protease that activates ENaC. A survey of the identification and use of various proteases that modulate ENaC activity is provided by Kleyman et al., J. Biol. Chem. 284:20447-20451, 2009. In principal, a compound suitable for use in this context may or may not act on ENaC directly, as long as contacting the cell with the compound has the effect of activating ENaC or increasing its expression without causing undesirable side effects.

[0050] To identify compounds suitable for increasing ENaC activity or expression according to this invention, the compounds can be screened using a cell line that constitutively expresses ENaC. Test compounds are combined with the cells, and transport or flux of sodium ion across the membrane of the cells is monitored. A test compound can be identified as having an ability to promote ENaC activity or increases ENaC expression if the cells treated with the compound show more transport of sodium ion than the same cells before or without treatment, or other cells that have been treated with a compound known not to modulate ENaC function.

Pharmaceutical compositions and their use

[0051] This invention provides pharmaceutical compositions, comprising one or more small molecule drugs, peptides, siRNA, antibodies, and other active agents of this invention that affect ENaC activity or expression.

[0052] Development of pharmaceutical composition for use in birth control or to treat inflammation involves obtaining a compound that inhibits transport of sodium ion through an epithelium sodium channel or reduces ENaC expression, as described above. The compound is then tested in a suitable animal model for the target disease (e.g., Example 3). Testing an agent for use in birth control comprises administering an ENaC inhibitor to a test subject when an embryo is present in the uterus of the subject following fertilization; and then determining whether the compound affects implantation of said embryo in the uterus of the subject. Testing an agent for use in treating an inflammatory condition comprises administering an ENaC inhibitor to a suitable animal model for the target disease, and then determining whether there has been a reduction in COX-2 activity, reduced production of prostaglandins such as PGE2, or a resolution of any of the symptoms or signs of the inflammatory condition.

[0053] Development of pharmaceutical composition for use in increasing the probability of embryo implantation in the uterine wall, or for increasing fertility involves obtaining a compound that promotes transport of sodium ion through an epithelium sodium channel or increases ENaC expression, as described above. The compound is then tested in a suitable animal model for pregnancy or implantation, where the rate of implantation is compared between treated animals and untreated controls.

[0054] Once an ENaC modulator compound has been selected and tested, an effective amount for treating the condition for which it is being formulated is combined that are both physiologically compatible at the treatment site, and compatible with the active agent.

[0055] For administration systemically (e.g., for the treatment of disseminated inflammatory disease), the active agent can be formulated in the form of a tablet for oral ingestion. For injection in or near a uterine horn, or at or near a site of inflammation, the active agent can be formulated for injection in a compatible buffer, and optionally lyophilized for transport and storage.

[0056] For administration from within the uterus, the composition may be formulated as a salve, gel, or cream. Possible excipients include a buffering agent, a wetting agent such as ethyl alcohol, a levigating agents such as mineral oil or glycerin, and/or a suspension agent such as carboxymethylcellulose or tragacanth. Other excipients may include components that give the topical formulation its desired consistency: such as specialty esters, fatty alcohols, ethoxylates, hard fats, stearic acids, and mono- and triglycerides. The composition may include a preservative to increase the shelf-life of the composition at room temperature.

[0057] The composition may also contain one or more agents that promote penetration of the active agent(s) into or through the mucosa. These may fall into chemical classes such as terpenes, non-ionic surfactants, azone, oleyl surfactants, fatty alcohols, and fatty acid esters. See, for example, US 2007/0269379 A1; US 2008/0152597 A1; US 2005/ 0070440 A1; US 2011/0196040 A1; and US 2005/0239724 A1.

[0058] For general formulation, preparation, storage, and use of pharmaceutical and cosmetic compositions according to this invention, the reader may refer to DRUG DELIVERY: PRINCIPLES AND APPLICATIONS (Wiley Series in Drug Discovery and Development), B Wang et al., 2005; PERCUTANEOUS ABSORPTION: DRUGS, COSMETICS, MECHANISMS, METHODS, RL Bronaugh et al., 2005; and the current edition of *Remington's Pharmaceutical Sciences*.

Glossary

[0059] The term "contragestion", is used in this disclosure to mean reducing the probability of pregnancy of a female following fusion of a female gamete with a male gamete in the uterus. Contragestion according to this invention can occur but is not limited to impairment of implantation of a fertilized embryo into a uterine wall in the subject. It is a method of birth control.

[0060] An "embryo" as the term is used in this disclosure means a vertebrate at any stage of development following fertilization and prior to birth.

[0061] An "inflammatory condition" is a condition in a mammalian subject wherein at least some of the symptoms and/or signs experienced by the subject are mediated by an inflammatory process. This includes both antigen-specific and non-specific responses to external or internal stimuli. Antigen-specific reactions include but are not limited to Type I hypersensitivity (allergy).

[0062] A sodium channel or ENaC "inhibitor" is a compound (for example, a small molecule drug, an siRNA, or an antibody) that is shown in the cell-based assays such as those provided in this disclosure to inhibit expression of said channel, and/or inhibit the ability of the channel to transport sodium ion across the membrane of the cell in which it is expressed.

[0063] A sodium channel or ENaC "activator" is a compound (for example, a small molecule drug, an siRNA, or an antibody) that is shown in the cell-based assays such as those provided in this disclosure to promote expression of said channel, and/or increase the ability of the channel to transport sodium ion across the membrane of the cell in which it is expressed.

[0064] A sodium channel or ENaC "modulator" is either an ENaC inhibitor or an ENaC activator.

[0065] A "small molecule drug" is a compound with pharmaceutical activity that is no more than 5,000 Da in size.

[0066] The term "antisense RNA" indicates a compound that acts to modulate the translation or other normal operation of a target RNA molecule by way of Watson-Crick base pairing between the compound and the target RNA. The compound itself may be but is not required to be ribonucleic acid.

[0067] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used in this disclosure, the term encompasses intact antibodies of

any species including human, antibody fragments, chimeras, humanized antibodies, single-chain variable regions, and equivalent proteins that include at least one antigen combining site of the desired specificity.

[0068] A "pharmaceutical excipient" is a liquid, semi-liquid or solid medium in which a biologically active agent in a pharmaceutical composition is administered to the treatment subject and delivered to the intended site of administration. The excipient is compatible with the active agent (i.e., it does not adversely interfere with the activity or stability of the agent), and is physiologically compatible with the subject (i.e., it is physiologically inert, sterile, and compounded under conditions suitable for human administration). Ingredients of a pharmaceutical excipient may include solvents, creams, gels, buffers, thickeners, emulsifying agents, preservatives, stabilizers, coloring agents, solid matrixes, coatings, and releasing agents.

[0069] An "effective dose" or "effective amount" of an active agent in a composition of this invention is an amount of the agent which (when given in the appropriate manner) is effective in achieving a treatment goal as indicated by the particular context. The amount is effective in achieving the goal in a substantial proportion or majority of subjects in the same situation and phenotype, and having the same condition or treatment goal as the subject actually being treated.

EXAMPLES

[0070] The illustrations provided below show that activation of ENaC in mouse endometrial epithelial cells by an embryo-released serine protease triggers Ca^{2+} influx that leads to PGE2 release, phosphorylation of transcription factor CREB, upregulation of cyclooxygenase 2 (COX-2), the enzyme important for prostaglandin (PG) production and implantation, and alteration of two COX-2-targeting microRNAs, miR-199a and miR-101.

[0071] Blocking or knocking down uterine ENaC in mice results in implantation failure accompanied with altered levels of uterine COX-2, miR-199a and miR-101. Uterine ENaC expression levels prior to *in vitro* fertilization (IVF) treatment were found to be significantly lower in women with implantation failure as compared with those with successful pregnancy.

[0072] These data demonstrate that ENaC plays a significant role in regulating PGE2 production and release required for embryo implantation. A defect in ENaC activity or expression may be a cause of miscarriage and low success rate in IVF.

Example 1. ENaC activation triggers Ca^{2+}-dependent and implantation-related signaling events

[0073] The immediate consequence of sodium ion influx on activation of ENaC is the influx of Na+ into the cell, resulting in membrane depolarization. This example uses a primary culture of mouse endometrial epithelial cells (EECs), to test whether a serine protease known to be released by invading embryo, trypsin, could induce membrane depolarization.

[0074] **Figure 1** illustrates ENaC-mediated signal transduction events and PGE2 release from mouse endometrial epithelial cells. **Figure 1(A)**, Trypsin-induced membrane depolarization assessed by voltage-sensitive fluorometric measurement in 1-day primary culture of mouse endometrial epithelial cells (EECs). Fluorescence photographs of EECs before (left) and after (right) treated with trypsin (20 μg/mL). Color coding bar showing increasing fluorescence intensity indicating membrane potentials from strongly hyperpolarized (purple) to strongly depolarized (red to white). The insets show higher resolution images of the circled areas. Bottom left: Calibration curve with measured changes in fluorescence intensity of DiBAC4(3) (% of baseline) as a function of membrane potential changes (calculated from changes in K-gluconate concentrations in the presence of 2 μM valinomycin). Bottom right: Summary of membrane potential changes (ΔVm) elicited by trypsin, trypsin after pretreatment with amiloride or aprotinin. (n = 15-31, ***p<0.001 compared with the trypsin-treated group).

[0075] **Figure 1(B)**, Representative time course changes in intracellular Ca^{2+} levels (indicated by 340/380 ratio of Fura-2) in EECs in response to trypsin (20 μg/mL) in the presence of amiloride (10 μM) or nifedipine (10 μM), with corresponding statistical analysis (n= 20- 81, ***p<0.001 compared with the trypsin-treated group). **Figure 1(C)**, Summary of trypsin-induced PGE2 release from 3-day culture of EEC (n = 4-6, **p<0.01, ***p<0.001). **Figure 1(D)**, Quantitative PCR (qPCR) analysis of COX-2 mRNA levels in EECs under different conditions (n=4, ***p<0.001). **Figure 1(E)**, Western blot analysis of protein levels of phosphorylated form of CREB (P-CREB). Data are presented as the ratio of P-CREB level to the level of β-tubulin (n=4, *p<0.05, **p<0.01, ***p<0.001). **Figure 1(F)**, Fluorescence labeling of P-CREB in EECs with DAPI labeled nuclei (Bars= 20 μm). **Figure 1(G)**, qPCR analysis of miR199a and miR101 levels in EECs (n=4, **p<0.01, ***p<0.001). All qPCR data are presented as relative values normalized with the absolute value under control condition (Ctrl). T: trypsin (20 μg/mL); Ami: amiloride (10 μM); Apr: aprotinin (20 μg/mL); Nif: nifidipine (10 μM); B: BAPTA/AM (50 μM). Data are means ± SEM.

[0076] As shown in **Figure 1(A)**, trypsin (20 μg/mL) was found to induce membrane depolarization (+22.24 ± 2.2 mV) in EECs, which was abrogated by either amiloride (10 μM), a specific blocker of ENaC, or aprotinin (20 μg/mL), a protease inhibitor. Given that Ca^{2+} mobilization, which is usually triggered by membrane depolarization, had been reported to facilitate COX-2- dependent production/release of PGE2, it was hypothesized that the serine protease-induced

ENaC mediated membrane depolarization could result in Ca^{2+} mobilization leading to PGE2 production/release. The effect of trypsin was tested on intracellular Ca^{2+} mobilization. As shown in **Figure 1(B)**, addition of trypsin (20 μg/mL) induced sustained Ca^{2+} elevation in EECs, which was inhibited by amiloride (10 μM) and aprotinin (20 μg/mL), suggesting the involvement of ENaC in mediating the effect of trypsin. The trypsin-induced Ca^{2+} rise was largely reduced in Ca^{2+}-free solutions suggesting Ca^{2+} influx through Ca^{2+} channels. Consistent with this notion, nifedipine (10 μM), a blocker of the voltage-dependent Ca^{2+} channel, abolished the trypsin-induced Ca^{2+} rise.

[0077] These results suggest that the ENaC-mediated membrane depolarization induced by the serine protease may activate voltage dependent Ca^{2+} channel leading to Ca^{2+} influx. To test this, L-type voltage-dependent Ca^{2+} channel mRNA expression and functional channel activities in EECs by the patch-clamp technique. **Figure 6(A)** shows PCR detection of L-type Ca^{2+} channel expression in mouse endometrial epithelial cells (EECs). **Figure 6(B)** is a patch-clamp recording of voltage-dependent Ca^{2+} channel activities (n=7). Left panel shows whole-cell currents elicited from -40 to 20 mV in a EEC before (Ctrl) and after addition of nifidepine (3μM). Right panel shows corresponding current-voltage curves showing voltage-dependent characteristic of L-type Ca^{2+} channel.

[0078] An ELISA kit was used to test whether trypsin could induce ENaC-dependent PGE2 release. It was found that trypsin (20 μg/mL) could induce a nearly 3-fold increase in PGE2 release from the epithelial culture, which could be attenuated by the pretreatment with either amiloride (10 μM) or aproptonin (20 μg/mL) (**Figure 1(C)**). Moreover, the trypsin induced PGE2 release from EECs was abolished by BAPTA/AM (50 μM), an intracellular calcium chelator, suggesting the involvement of intracellular Ca^{2+} in triggering PGE2 release. Taken together, the results suggest that the activation of ENaC by the serine protease may result in membrane depolarization, which in turn leads to Ca^{2+} influx, resulting in PGE2 release from EECs.

[0079] It has been suggested that persistent elevation of PGs is required for decidualization. ENaC may also play a role in PGs production during implantation, for example, by regulating the expression of COX-2, a key enzyme in PGs synthesis. As shown by the quantitative PCR result (**Figure 1(D)**), a significantly elevated mRNA level of COX-2 was found in the EECs after 15 min treatment with trypsin (20 μg/mL), which was abolished by pre-treating the cells with either amiloride (10 μM) or nifidipine (10 μM). These results suggest that up-regulation of COX-2 in EECs may be induced by ENaC activation-dependent Ca^{2+} influx. In this regard, transcription of COX-2 has been reported to be activated by the phosphorylation of cAMP/Ca^{2+} response element binding protein (CREB), a transcription factor known to regulate PGs production.

[0080] Although ENaC has never been implicated in the regulation of gene transcription, the observed Ca^{2+} influx upon protease-induced ENaC activation prompted us to test whether ENaC activation could lead to CREB phosphorylation, thereby regulating COX-2 expression and PGs production in EECs. Indeed, Western blot analysis showed that the phosphorylated form of CREB

(P-CREB) was significantly increased in EECs after the treatment with trypsin (20 μg/mL), which was attenuated by the pretreatment with amiloride (10 μM) or nifidipine (10 μM) (**Figure 1(E)**). Immunofluorescence also showed an increased accumulation of P-CREB in the nuclei of cultured EECs after treatment with trypsin, which was blocked by amiloride (**Figure 1(F)**). These results suggested that ENaC activation could regulate transcriptional activities of COX-2.

[0081] It has also been suggested that COX-2 can be regulated at post-transcriptional level by microRNAs (miRNAs), which are small non-coding RNAs that may interfere with target protein translation. In the present study, two miRNAs, miR-199a and miR-101, which had been shown to suppress COX-2 and be critically involved in embryo implantation, were significantly reduced in the trypsin-treated EECs, and this reduction could be reversed by either amiloride or nifidipine (**Figure 1(G)**), suggesting post-transcriptional regulation of COX-2 via miRNAs by ENaC activation. Taken together, the results indicate that the persistently high levels of PGs required for decidualization and implantation may be achieved by the activation of ENaC with subsequent Ca^{2+} influx, which may upregulate COX-2 expression either by CREB-mediated transcriptional activity or by miR-199a and miR-101 targeting COX-2.

Example 2. ENaC activation induces decidualization *in vitro*

[0082] This example tests whether the activation of endometrial ENaC by the serine protease could indeed lead to stromal cell decidualization.

[0083] **Figure 2** shows involvement of ENaC in trypsin-induced decidualization in endometrial epithelial stromal cell co-culture. **Figure 2(A)**, Bright-field photographs (bars=50 μm) show the morphology of the cocultured stromal cells before (Ctrl) and after treatment with trypsin (T, 20 μg/mL). Fluorescence photographs (bars=20 μm) show the anti-desmin signal on the co-cultured stromal cells before and after trypsin treatment, in the absence or presence of amiloride (Ami, 10 μM) or PGE2 (10 μM). **Figure 2(B)**, Summary of trypsin-induced intracellular cAMP increase in the co-culture, which could be inhibited by amiloride (Ami, 10 μM) and EP4 receptor antagonist AH2384 (AH, 10 μM). n= 6, *$p<0.05$, ***$p<0.001$.

[0084] An epithelial-stromal co-culture was established. As shown in **Figure 2(A)**, the treatment of the co-culture with trypsin (20 μg/mL, 4-6h) induced significant morphological changes of the stromal cells from elongated cells into giant, polygonal and multi-nuclei cells, as characterized for decidual cells *in vitro*. In addition, the expression of desmin, a reported marker of decidual cells, was found up-regulated in the trypsin-treated co-culture, as indicated by the immunostaining results (**Figure 2(A)**). Pretreatment with amiloride (10 μM) attenuated the effect of trypsin both in inducing morphological change and desmin expression on the stromal cells, while the inhibitory effect of amiloride could be reversed by adding PGE2 (10 μM) to the co-culture (**Figure 2(A)**).

[0085] These results suggested that the serine protease could induce stromal decidualization *in vitro* by activating ENaC in the endometrial epithelial cells, an action that could be mimicked by PGE2. As a decidualizing molecule, PGE2 is believed to activate EP2/EP4 receptors on stromal cells, which in turn leads to the accumulation of intracellular cAMP ([cAMP]i) and thus decidualization.

[0086] The next experiment tested whether ENaC activation could lead to EP2/EP4 receptor-mediated elevation of cAMP in EECs. Trypsin (20 μg/mL, 20 min) was added to the EEC-stroma co-culture and found elevated [cAMP]i level, which could be attenuated by pretreatment with amiloride (10 μM) (**Figure 2(B)**). Consistent with the morphological result (**Figure 2(A)**), adding PGE2 could also reverse the inhibitory effect of amiloride on the trypsin-induced [cAMP]i in the co-culture. Moreover, EP4 receptor antagonist, AH2384 abolished the enhancing effect of trypsin on [cAMP]i in the co-culture, confirming the involvement of the PG receptor in mediating the effect of ENaC activation on intracellular cAMP production. Taken together, the results obtained *in vitro* have clearly demonstrated that the activation of ENaC by the serine protease could lead to stromal decidualization involving PGE2 and it receptor.

Example 3. Interfering with ENaC expression/function impairs embryo implantation in mice

[0087] This example tests whether interfering with ENaC function in vivo could impair implantation/decidualization.

[0088] **Figure 3** shows requirement of ENaC for embryo implantation in vivo. **Figure 3(A)**, Effect of intrauterine injection (on day 3 post mating) of amiloride, EIPA or aprotinin on implantation rate in mice, indicated by the number of implanted embryos observed in each uterine horn on day 7. Ctrl: control; Amil: amiloride (0.01-5 mM); EIPA: ethylisopropyl-amiloride (0.01-0.1 mM); Apr: aprotinin (200 μg/mL). (n=8-32, ***$p<0.001$ compared with Ctrl. **Figure 3(B)**, Western blotting data showing the effectiveness of in vivo knockdown of ENaCα by intrauterine injection of siRNAENaCα (n=4, **$p<0.01$ compared with siRNANC). **Figure 3(C)**, Effect of ENaCα knockdown on implantation rate in mice with a photograph showing implantation sites (arrowed) in the control uterine horn (siRNANC) as compared with the siRNAENaCα treated one. **Figure 3(D)**, H-E pictures showing the effect of ENaCα knockdown on decidualization in mice on day 5, with less decidual cells observed as compared with the control. **Figure 3(E)**, Effect of ENaCα knockdown on uterine miRNA levels, measured by qPCR, on day 4 and day 7 (n=4, **$p<0.01$). **Figure 3(F)**, Western blot analysis showing the effect of ENaCα knockdown on the uterine expression of COX-2 on day 4 and day 7 (n=4, **$p<0.01$).

[0089] As shown in **Figure 3**, intrauterine injection with amiloride (100 μM-5 mM) on day 3 of pregnancy could induce dose-dependent reduction in the number of implanted embryos in the amiloride-treated uterine horns compared with that in the vehicle-treated control horns on day 7 of

pregnancy (**Figure 3(A)**). To exclude possible non-specific effect of amiloride on sodium-proton exchanger (NHE), ethyl isopropyl amiloride (EIPA), a specific blocker of NHE was also applied. Unlike amiloride, injection with EIPA (100 μM) showed no significant effect on implantation rate in mice (**Figure 3(A)**).

[0090] To confirm that serine proteases (to which ENaC is sensitive) are required for implantation, aprotinin was applied to inhibit serine proteases in the pregnant mice. Similar to amiloride, aprotinin (200 μg/mL) was also found to significantly reduce the number of implanted embryos (**Figure 3(A)**). The impaired implantation resulted from interfering with ENaC activation, either by protease inhibitor or ENaC blocker, indicates the requirement of ENaC in the process of embryo implantation. To further confirm that ENaC is required for implantation, the expression of ENaC was manipulated in vivo using siRNAs targeting ENaCα (siRNAENaCα). Both siRNAENaCα (20 pmole/horn) and non-silencing siRNAs for negative control (siRNANC, 20 pmole/horn) were applied on day 3 of pregnancy by intrauterine injection.

[0091] The expression of ENaCα protein in the uteri was checked on day 4 by Western blotting (**Figure 3(B)**), showing significantly reduced protein level of ENaCα in the uteri injected with siRNAENaCα compared with those injected with siRNANC. The implanted embryo number counted on day 7 of pregnancy in the ENaC-knockdown uterine horns was found to be significantly reduced compared with the control (**Figure 3(C)**).

[0092] Using hematoxylin-eosin (H-E) staining, the morphology of the uteri was evaluated. It was observed that on day 5, the number of cells with vacuolated cytoplasm and condensed chromatin (typical morphology of decidual cells) was significantly reduced in the siRNAENaCα-treated uteri as compared with the control uteri (**Figure 3(D)**), indicating impaired decidualization upon ENaC knockdown. Thus, the in vivo ENaC knockdown experiment confirms the results obtained using amiloride or aprotinin, demonstrating the requirement of ENaC in implantation and decidualization.

[0093] The morphology of the uteri was observed on day 5. **Figure 7(A)** (H-E staining) show the effect of ENaCα knockdown on decidualization in mice. The number of cells with typical morphology of decidual cells was significantly reduced in the $siRNA_{ENaC\alpha}$-treated uteri, compared with the control uteri, indicating impaired decidualization upon ENaC knockdown. This confirms the results obtained using amiloride or aprotinin, demonstrating the requirement of ENaC activity for implantation.

[0094] A role of ENaC in implantation is also demonstrated by the effect of amiloride and ENaC knockdown on implantation markers, HoxA10, IgF2 and LIF. **Figure 7(B)** show results of a qPCR assay. Interference with ENaC function/expression substantially reduced the expression of these markers. This is consistent with the hypothesis that ENaC activation is an upstream event that modulates implantation.

[0095] Previous studies have demonstrated a dynamic uterine expression pattern of COX-2 in peri-implantation in mice. The uterine COX-2 has been found to be at a low to undetectable level on day 4 before embryo attachment, with increasing expression levels after the attachment up to day 6.

[0096] The impaired implantation in ENaC knockdown mice was investigated to determine if it was associated with a disrupted COX-2 expression levels. The expression levels of COX-2, miR-101 and miR-199a was evaluated in the siRNA-treated uteri of day 4 (before implantation) and day 7 (after implantation). As shown in **Figure 3(E)**, the expression of both miR101 and miR199a were significantly reduced on day 4, while enhanced on day 7 in siRNAENaCα treated uteri as compared with the control. Since these miRNAs are known to suppress COX-2, the reduced and increased levels of the miRNAs on day 4 and 7 upon ENaC knockdown, respectively, were consistent with the observed COX-2 protein levels, which were increased on day 4 but significantly reduced on day 7 in siRNAENaCα treated uteri (**Figure 3(F)**).

[0097] The altered miRNAs/COX-2 expression in ENaC knockdown animals with implantation failure confirms the involvement of ENaC in regulating COX-2/PGE2 during implantation. The altered miR101 and miR199a levels seen in siRNAENaCα treated uteri indicate the possible involvement of ENaC in epigenetic regulation of COX-2 expression and thus PGE2 production during decidualization/implantation. Thus, the findings also reveal a previously unsuspected role of ENaC in epigenetic regulation, especially where miRNAs are concerned, although the detailed mechanisms await further investigation.

Example 4. Reduced uterine ENaC expression in patients with IVF failure

[0098] Despite major improvement in assisted reproduction techniques (ART), the clinical pregnancy rate per embryo transfer (ET) in fresh ART cycles, or successful rate of IVF, remains at a low end of 31%. Since ENaC is required for embryo implantation as demonstrated presently in mice, alteration in ENaC expression or function may be a cause of implantation failure during IVF in humans.

[0099] To evaluate this, the expression levels of ENaC was determined in human endometrial samples obtained prior to IVF treatment and compared between women with successful (n=16) and failed (n=16) pregnancy. The demographic and clinical data of the patients showed no significant differences in age, ovarian hormonal profiles, endometrial thickness and oocyte quality between the two groups.

[0100] **Figure 4** presents a comparison of endometrial expression of ENaCα between women undergoing IVF treatment with successful and failed pregnancy. Western blot analysis of protein levels of ENaCα from endometrial biopsy samples collected before the cycle of IVF-ET treatment.

Data are presented as the ratio of ENaCα subunit levels to the level of β-actin (n=16 in each group *p<0.05)

[0101] As shown in **Figure 4**, the endometrial samples from women with failed pregnancy after IVF-ET showed a significantly lower averaged level in ENaCα subunit expression as compared with that from women with successful pregnancy. Five out of 16 patients with failed pregnancy showed a very low endometrial ENaCα expression which was well below the lowest expression level found in those with successful pregnancy.

[0102] **Figure 8** provides a statistical analysis of patients' clinical parameters. There were no significant differences in age, ovarian hormonal profiles, endometrial thickness and oocyte quality between the two groups.

[0103] **Figure 9** is a comparison of endometrial expression of ENaC between women undergoing IVF treatment with successful and failed pregnancy. Western blot analysis was done to determine protein levels of ENaCα from endometrial biopsy samples collected before the cycle of IVF-ET treatment. The data are presented as the ratio of the level of ENaC subunits α, β, and γ compared with the level of β-Actin. n=16 (successful pregnancy) and 17 (failed pregnancy). **: P<0.01; ***: P<0.001; ns: P>0.05. The endometrial samples from women with failed pregnancy after IVF-ET showed a significantly lower average level of ENaCα and ENaCγ subunit expression, compared with women with successful pregnancy.

[0104] These results, together with the results obtained from mice *in vitro* and in vivo, suggest that abnormal ENaC expression could lead to implantation failure in humans. Since ENaC expression is subject to regulation by ovarian hormones, its normal expression pattern may be altered during IVF with ovarian overstimulation, which may contribute to the low pregnancy rate in IVF. It is also noted that mutations of ENaC are well-documented, with abnormally enhanced ENaC function as observed in Liddle syndrome and abnormally reduced ENaC activity as observed in pseudohypoaldosteronism type 1 syndrome. Thus, a defect in ENaC, either expression or function due to its mutations, may be one of the underlying mechanisms for spontaneous miscarriage and implantation failure during IVF

Example 5. Interfering with ENaC expression/function is anti-inflammatory

[0105] This example was conducted using the human airway epithelial cell line, HBE as a model for inflammatory disease.

[0106] **Figure 10(A)** is a Western blot detecting phosphorylation of CREB. The cells were treated with the ENaC activator trypsin (20 μg/ml). The trypsin increased phosphorylation of transcription factor CREB in a time-dependent manner.

[0107] **Figure 10(B)** shows results of another experiment in which ENaC was activated by mechanical stimulation (shaking for 30 minutes). Activating cells in this way also led to CREB phosphorylation. The phosphorylation was abolished by the ENaC inhibitor amiloride (10 μM).

[0108] cAMP response element-binding protein (CREB) is a cellular transcription factor that binds cAMP response elements (CRE). CREB activity depens on phosphorylation, and regulates expression of COX-2. Prostaglandin-endoperoxide synthase 2 (COX-2) is an enzyme implicated in the biosyntehsis of inflammatory mediators such as prostaglandins (PGD2, PGE2, $PGF_{2\alpha}$), prostacyclin (PGI2), and thromboxane A2. Since CREB facilitates COX-2 dependent production and release of mediators such as PGE2, inhibiting ENaC activity would decrease the production of such mediators, thereby having an anti-inflammatory effect.

[0109] The results shown here are similar to what was observed with the uterine endometrial epithelial cells (Example 3). Clearly, ENaC is involved in regulation of the COX-2 pathway in tissues outside the uterus. The data confirm that inhibition of ENaC either locally or systemically can be used as a means for treating inflammatory conditions.

Example 6. Methods used in preceding examples

[0110] **Mice and intra-uterine injection.** Female ICR mice were purchased from the Laboratory Animal Service Centre of the Chinese University of Hong Kong. All animal experiments were conducted in accordance with the university guidelines on animal experimentation and approval by the Animal Ethic Committee of the Chinese University of Hong Kong was obtained for all related procedures. The day a plug was found after mating was designated as day 1 post mating. Intrauterine injection was done on day 3 around 18:00-20:00 pm. Uteri were exposed during abdominal surgery under general anesthesia. 20 μL PBS was injected into the lumen of each uterine horn close to the utero-tubal junction.

[0111] **Patients and endometrial sample collection.** Woman patients, who were diagnosed of infertility without hydrosalpinx syndrome and sought IVF treatment at Women's Hospital of School of Medicine of Zhejiang University, were recruited with written consents. The endometrial samples were collected during endometrium biopsy examination on day 21 (mid-secretory phase) of menstrual cycle before IVF-ET treatment. Western blotting assay was performed on 16 samples each randomly selected from the sample pools of successful pregnancy and failed pregnancy, according to the pregnancy outcome after IVF-ET.

[0112] **Primary culture.** Endometrial epithelial were isolated and cultured as previously described. Briefly, uteri were obtained from immature ICR mice, sliced longitudinally and treated with 6.5 mg/mL trypsin and 25 mg/mL pancreatin in PBS at 0°C for 60 min and at room temperature for another 45 min. The uteri were transferred to fresh PBS and gently shaken by hands for 30 sec to release epithelial cells. After collecting the released epithelial cells, the remaining

uterine tissue was washed with fresh PBS twice, incubated in 0.1% trypsin at 37 °C for further 15 min, transferred to fresh PBS and shaken for 30 sec to release stromal cells For the cocultures, the epithelial cells were mixed with the stromal cells at ratio of 1:1 and incubated in DMEM/F-12 with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, 100 IU/mL penicillin, and 10 μg/mL streptomycin at 37 °C.

[0113] **Patch-clamp.** A bath solution containing (mM): Na-gluconate 145, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 2, glucose 5.5 and Hepes 10 (pH 7.4) and a pipette solution containing (mM): K-gluconate 135, KCl 10, NaCl 6, $MgCl_2$ 2 and Hepes 10 (pH 7.2) were used for ENaC currents. To record Ca^{2+} channel currents, cells were bathed in the solution containing (mM): NaCl 130, $BaCl_2$ 10, $CaCl_2$ 0.2, TEACl 3, $MgCl_2$ 0.6, $NaHCO_3$ 14, NaH_2PO_4 1, Hepes buffer 33, glucose 5.5 (pH 7.2) with pipettes filled with a solution containing (mM): NaCl 10, TEACl 100, $MgSO_4$ 2, EGTA 5.5, $CaCl_2$ 0.5 and Hepes 10 (pH 7.2).

[0114] **Membrane potential measurement.** Epithelial cells on cover-slips were washed with a bath solution containing (mM): NaCl 135, KCl 5.8, MgCl2 1.2, CaCl2 2.5, Hepes 10 and glucose 5 (pH 7.4), and then loaded with the voltage-sensitive dye DiBAC4(3) (1 μM). Fluorescence (495/520 nm excitation/emission) was monitored and calculated to membrane potential change by adding K_+-gluconate (5-60 mM) in the presence ofvalinomycin (2 μM), as previously described.

[0115] **Intracellular calcium measurement.** Epithelial cells on cover-slips were loaded with fura2 (3 μM) at 37 °C for 30 min. Fluorescence excited at 340 and 380 nm was monitored

[0116] **PGE2 measurement.** Epithelial cells were grown on Transwell-Col membranes (0.4 μm) for 3 days. FBS concentration in the culture medium was reduced to 1% 12 hours before the experiment. Immediately before starting the experiment, the cells were changed into no-FBS media. After treatment, cell-free supernatant was collected and PGE2 content was measured using an EIA kit (Cayman Chemical).

[0117] **cAMP measurement.** After treatment, co-cultured cells were lysed in 0.1 mM HCl and 0.1% Triton-200 for 10 min. cAMP content was measured using an ELISA kit (Assay Design).

[0118] **Immunostaining.** Cells on cover-slips were fixed with 4% PFA in PBS for 15 min permeablized with 1% Triton- 200 in PBS for 5 min and incubated in PBS containing 5% rabbit serum for 30 min for blocking. Primary antibodies against desmin (Abcam, 1: 100), and P-CREB (Cell Signaling, 1:100) were applied on cells for overnight at 4°C.

[0119] **In vivo RNAi.** Stealth™ RNA duplex oligoribonucleotides (AAA GCA AAC UGC CAG UAC AUG C and GCA UGA UGU ACU GGC AGU UUG CUU U targeting ENaCα (siRNAENaCα), Stealth™ RNAi Negative Control Lo GC Duplex (siRNANC), and Lipofectamine™ 2000 were purchased from Invitrogen. The intrauterine injection surgery was as described above done on day 3 of pregnancy. 20 pmole siRNAENaCα or siRNANC combined with lipofectamine was injected to each uterine horn. siRNAENaCα and siRNANC were injected respectively to each uterine horn of the pair in the same mouse.

[0120] **Statistics.** Data are presented as mean ± SEM (n is the number of tissue preparations, or cells, or experiment times). For two groups of data, two-tail Student's t test was used. For three or more groups, data were analyzed by one-way ANOVA with Dunnett's post hoc test. A value of $p<0.05$ was considered to be statistically significant.

SEQUENCES

AAA GCA AAC UGC CAG UAC AUG C (SEQ ID NO:1)

GCA UGA UGU ACU GGC AGU UUG CUU U (SEQ ID NO:2)

amiloride-sensitive sodium channel subunit alpha [Mus musculus].
ACCESSION NP_035454 XP_987354

mRNA: SEQ ID NO:3

Underlined sequence is siRNA

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   1 agtcacagcc cagccacacc tggagccggg agcaggaggc agctccggcc tcctgcaacc
  61 cacggtcccc gaggcagaga aggaggtagc agggagctgg aggccagggc tagagagcct
 121 agagaagagg acccaggagg agatagggaa ggcaggaaag gaagtgaggc aggatcagag
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 301 cagacagggt gcctgctgtg gagacccagg gaggcgctag cgggcaaacg aaggtggcct
 361 tcgctgtgac cacttcgctc tgtggccact ccagtgaagc tccgtgctgc ctggttggcc
 421 ccaactccag aaggtcagct ggctcctgga gaggtggagg agggtgggag gactgaggaa
 481 gagggaactc agcgtgggat gcgggcaccg tcacggacgg ccccattctg cctccatact
 541 aatgatgctg gaccacacca gagcccctga gctcaacctt gacctagacc ttgacgtctc
 601 caactcaccg aagggatcca tgaagggcaa caatttcaag gagcaagacc tttgtcctcc
 661 tctgcccatg caaggactgg gcaaggggga caagcgtgaa gaacaggcgc tgggcccgga
 721 accctcagag ccccggcagc ccaccgagga ggaggaggca ctgatcgagt tccaccgctc
 781 ctaccgggag ctcttccagt tcttctgcaa caataccacc atccacggtg ccatccgcct
 841 ggtgtgctcc aagcacaacc gcatgaagac ggccttctgg gcggtgctct ggctctgcac
 901 cttcggcatg atgtactggc agtttgcttt gctgttcgag gaatacttca gctaccccgt
 961 gagtctcaac atcaacctca attcggacaa gctggtcttc cctgccgtca ctgtgtgcac
1021 ccttaatcct tacagataca ctgaaattaa agaggatctg gaagagctgg accgcatcac
1081 ggaacagacg ctttttgacc tgtacaaata caactcttcc tacactcgcc aggctggggg
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1201 cacaccacct ccgcccaatc ccgcccgctc ggcgcgcagc gcgtcctcca gtgtacgcga
1261 caacaatccc caagtggaca ggaaggactg gaaaatcggc ttccaactgt gcaaccagaa
1321 caaatcagac tgcttctacc agacatactc atccggggtg gatgccgtga gagaatggta
1381 ccgcttccat tacatcaaca ttctgtccag actgcccgac acctcgcctg ctctagagga
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1501 gaattattct cagttccacc accccatgta tgggaactgc tacactttca acaacaagaa
1561 caactccaat ctctggatgt cttccatgcc tggagtcaac aatggtttgt ccctgacact
1621 gcgcacagag cagaatgact tcatccccct gctgtccaca gtgacggggg ccagggtgat
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1861 acagcaggtg tgcattcact cctgcttcca ggagaacatg atcaagaagt gtggctgtgc
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2161 cacgatcaac aacaaaagaa acggagttgc taaactcaac atcttcttca aagagctgaa
2221 ctataaaact aattcggagt ctccctctgt cacgatggtc agcctcctgt ccaacctggg
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2521 cacgacccct cccctggccc tgacagcccc tccacctgcc tatgctaccc taggcccctc
2581 tgcctctcca ctggactcgg ctgtgcctgg ctcttctgcc tgtgctccgg ccatggcact
2641 ctgagagagg agagtgctcc tctcacccag gccagtgctc ctgtcacttc agcacatctt
2701 ccacagctgc ccagctgtct ttggtgtgtc ccggaggaac aggctaagca aggggcccag
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```
2761 gaagttgtcc agaggacagg ggctaatgag ctgctcagag ctgccctgcc cctgcttctg
2821 aacactgctt tccacacaag cacgggcaag tccccтttac ccttggatca gccaagccag
2881 acttggagct ttgacaagga acgttcccgg gaaacgacca aacgaaccga acacatataa
2941 acaaggcaca gagaagtggc cacagccttc ccaccccacg accagagact ggcctggcct
3001 cactgctttc aaggacacag atgtctgcta cccctcttga acttgggtgg ggaaccccac
3061 ccaaaagccc ccttgttagc tctttggcaa ttctccttcc ctcactcctc agggtggggg
3121 ctagagtaag cctgacatcc tcctccattc tcaagactct ctctctttca tttggtaccc
3181 tgtaccccag tgcctctgcg tcgcctcctt cttgtgtgcc ttctgagctg tttcttcagc
3241 ctagaaactc cctgctcaaa ggcacctttg cttttgtgaa ctcgttcacc ctatcctgtc
3301 tcccccagga ttgccccccc ctcccctcac ccccacagca tgctgtatta gatgctcaca
3361 ttcttttgtg tccatctccc tgggtagact gaactgtgct cagggatgag ctttgctcat
3421 ttttgtatcc ttccgttcta gcccagtatc ccacttggac caggtaggca gatactcaat
3481 aaatgcttgt tccatcaaaa aaaaaaaaaa aaaaa
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Protein: SEQ ID NO:4

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  61 pseprqptee eealiefhrs yrelfqffcn nttihgairl vcskhnrmkt afwavlwlct
 121 fgmmywqfal lfeeyfsypv slninlnsdk lvfpavtvct lnpyryteik edleeldrit
 181 eqtlfdlyky nssytrqagg rrrstrdlrg alphplqrlr tppppnpars arsasssvrd
 241 nnpqvdrkdw kigfqlcnqn ksdcfyqtys sgvdavrewy rfhyinilsr lpdtspalee
 301 ealgsfiftc rfnqapcnqa nysqfhhpmy gncytfnnkn nsnlwmssmp gvnnglsltl
 361 rteqndfipl lstvtgarvm vhgqdepafm ddggfnvrpg vetsismrke aldslggnyg
 421 dctengsdvp vknlypskyt qqvcihscfq enmikkcgca yifypkpkgv efcdylkqss
 481 wgycyyklqa afsldslgcf skcrkpcsvt nyklsagysr wpsvksqdwi femlslqnny
 541 tinnkrngva klniffkeln yktnsespsv tmvsllsnlg sqwslwfgss vlsvvemael
 601 ifdllvitli mllhrfrsry wspgrgarga revastpass fpsrfcphpt spppslpqqg
 661 ttpplaltap ppayatlgps aspldsavpg ssacapamal
```

Amiloride-sensitive sodium channel subunit beta [Homo sapiens]
Epithelial Na(+) channel subunit beta
Beta-ENaC
640 aa protein
Accession: P51168.2 GI: 8928561

mRNA: SEQ ID NO:5

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   1 gtgcttcccc gcccctgaac ctgctccctc ccagtcggtc tcgccgcgct cgccgggtgt
  61 cccagtgtca ccaacactcg gccgccgccg ccagcttggc gcgcaccgcc gcctccgcca
 121 ccgccgacag cgcgcatcct ccgtgtcccc gctccgccgc ccgagcaggt gccactatgc
 181 acgtgaagaa gtacctgctg aagggcctgc atcggctgca gaagggcccc ggctacacgt
 241 acaaggagct gctggtgtgg tactgcgaca acaccaacac ccacggcccc aagcgcatca
 301 tctgtgaggg gcccaagaag aaagccatgt ggttcctgct caccctgctc ttcgccgccc
 361 tcgtctgctg gcagtggggc atcttcatca ggacctactt gagctgggag gtcagcgtct
 421 ccctctccgt aggcttcaag accatggact tccctgccgt caccatctgc aatgctagcc
 481 ccttcaagta ttccaaaatc aagcatttgc tgaaggacct ggatgagctg atggaagctg
 541 tcctggagag aatcctggct cctgagctaa gccatgccaa tgccaccagg aacctgaact
 601 tctccatctg gaaccacaca cccctggtcc ttattgatga acggaacccc caccacccca
 661 tggtccttga tctctttgga gacaaccaca atggcttaac aagcagctca gcatcagaaa
 721 agatctgtaa tgcccacggg tgcaaaatgg ccatgagact atgtagcctc aacaggaccc
 781 agtgtacctt ccggaacttc accagtgcta cccaggcatt gacagagtgg tacatcctgc
 841 aggccaccaa catctttgca caggtgccac agcaggagct agtagagatg agctaccccg
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 961 cgtccatctt ctaccctcac tatggcaact gttacatctt caactggggc atgacagaga
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1081 gccaggaaga ctacgtcccc ttccttgcgt ccacggccgg ggtcaggctg atgcttcacg
1141 agcagaggtc ataccccttc atcagagatg agggcatcta cgccatgtcg gggacagaga
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1561 ggccttctga ggcctccgag gactggattt tccacgtctt gtctcaggag cgggaccaaa
1621 gcaccaatat caccctgagc aggaagggaa ttgtcaagct caacatctac ttccaagaat
1681 ttaactatcg caccattgaa gaatcagcag ccaataacat cgtctggctg ctctcgaatc
1741 tgggtggcca gtttggcttc tggatggggg gctctgtgct gtgcctcatc gagtttgggg
1801 agatcatcat cgactttgtg tggatcacca tcatcaagct ggtggccttg gccaagagcc
1861 tacggcagcg gcgagcccaa gccagctacg ctggcccacc gcccaccgtg gccgagctgg
1921 tggaggccca caccaacttt ggcttccagc ctgacacggc cccccgcagc cccaacactg
1981 ggccctaccc cagtgagcag gccctgccca tcccaggcac cccgcccccc aactatgact
2041 ccctgcgtct gcagccgctg gacgtcatcg agtctgacag tgagggtgat gccatctaac
2101 cctgcccctg cccaccccgg gcggctgaaa ctcactgagc agccaagact gttgcccgag
2161 gcctcactgt atggtgccct ctccaaaggg tcgggagggt agctctccag gccagagctt
2221 gtgtccttca acagagaggc cagcggcaac tggtccgtta ctggccaagg gctctgtaga
2281 atcacggtgc tggtacagga tgcaggaata aattgtatct tcacctggtt cctaccctcg
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2341 tccctacctg tcctgatcct ggtcctgaag acccctcgga acaccctctc ctggtggcag
2401 gccacttccc tcccagtgcc agtctccatc caccccagag aggaacaggc gggtgggcca
2461 tgtggttttc tccttcctgg ccttggctgg cctctggggc aggggtggtg gagagatgga
2521 agggcatcag gtgtagggac cctgccaagt ggcacctgat ttactctaga aaataaaagt
2581 agaaaatact gagtcca
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Protein: SEQ ID NO:6

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   1 MHVKKYLLKG LHRLQKGPGY TYKELLVWYC DNTNTHGPKR IICEGPKKKA MWFLLTLLFA
  61 ALVCWQWGIF IRTYLSWEVS VSLSVGFKTM DFPAVTICNA SPFKYSKIKH LLKDLDELME
 121 AVLERILAPE LSHANATRNL NFSIWNHTPL VLIDERNPHH PMVLDLFGDN HNGLTSSSAS
 181 EKICNAHGCK MAMRLCSLNR TQCTFRNFTS ATQALTEWYI LQATNIFAQV PQQELVEMSY
 241 PGEQMILACL FGAEPCNYRN FTSIFYPHYG NCYIFNWGMT EKALPSANPG TEFGLKLILD
 301 IGQEDYVPFL ASTAGVRLML HEQRSYPFIR DEGIYAMSGT ETSIGVLVDK LQRMGEPYSP
 361 CTVNGSEVPV QNFYSDYNTT YSIQACLRSC FQDHMIRNCN CGHYLYPLPR GEKYCNNRDF
 421 PDWAHCYSDL QMSVAQRETC IGMCKESCND TQYKMTISMA DWPSEASEDW IFHVLSQERD
 481 QSTNITLSRK GIVKLNIYFQ EFNYRTIEES AANNIVWLLS NLGGQFGFWM GGSVLCLIEF
 541 GEIIIDFVWI TIIKLVALAK SLRQRRAQAS YAGPPPTVAE LVEAHTNFGF QPDTAPRSPN
 601 TGPYPSEQAL PIPGTPPPNY DSLRLQPLDV IESDSEGDAI
```

Amiloride-sensitive sodium channel subunit alpha [Homo sapiens]
Epithelial Na(+) channel subunit alpha
Alpha-ENaC
669 aa protein
Accession: P37088.1 GI: 585966

mRNA: SEQ ID NO:7

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  61 cccagtgtca ccaacactcg gccgccgccg ccagcttggc gcgcaccgcc gcctccgcca
 121 ccgccgacag cgcgcatcct ccgtgtcccc gctccgccgc ccgagcaggt gccactatgc
 181 acgtgaagaa gtacctgctg aagggcctgc atcggctgca gaagggcccc ggctacacgt
 241 acaaggagct gctggtgtgg tactgcgaca acaccaacac ccacggcccc aagcgcatca
 301 tctgtgaggg gcccaagaag aaagccatgt ggttcctgct caccctgctc ttcgccgccc
 361 tcgtctgctg gcagtggggc atcttcatca ggacctactt gagctgggag gtcagcgtct
 421 ccctctccgt aggcttcaag accatggact tccctgccgt caccatctgc aatgctagcc
 481 ccttcaagta ttccaaaatc aagcatttgc tgaaggacct ggatgagctg atggaagctg
 541 tcctggagag aatcctggct cctgagctaa gccatgccaa tgccaccagg aacctgaact
 601 tctccatctg gaaccacaca cccctggtcc ttattgatga acggaacccc caccacccca
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 721 agatctgtaa tgcccacggg tgcaaaatgg ccatgagact atgtagcctc aacaggaccc
 781 agtgtacctt ccggaacttc accagtgcta cccaggcatt gacagagtgg tacatcctgc
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2461 tgtggttttc tccttcctgg ccttggctgg cctctggggc aggggtggtg gagagatgga
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2581 agaaaatact gagtcca
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Protein: SEQ ID NO:8

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   1 MEGNKLEEQD SSPPQSTPGL MKGNKREEQG LGPEPAAPQQ PTAEEEALIE FHRSYRELFE
  61 FFCNNTTIHG AIRLVCSQHN RMKTAFWAVL WLCTFGMMYW QFGLLFGEYF SYPVSLNINL
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 121 NSDKLVFPAV TICTLNPYRY PEIKEELEEL DRITEQTLFD LYKYSSFTTL VAGSRSRRDL
 181 RGTLPHPLQR LRVPPPPHGA RRARSVASSL RDNNPQVDWK DWKIGFQLCN QNKSDCFYQT
 241 YSSGVDAVRE WYRFHYINIL SRLPETLPSL EEDTLGNFIF ACRFNQVSCN QANYSHFHHP
 301 MYGNCYTFND KNNSNLWMSS MPGINNGLSL MLRAEQNDFI PLLSTVTGAR VMVHGQDEPA
 361 FMDDGGFNLR PGVETSISMR KETLDRLGGD YGDCTKNGSD VPVENLYPSK YTQQVCIHSC
 421 FQESMIKECG CAYIFYPRPQ NVEYCDYRKH SSWGYCYYKL QVDFSSDHLG CFTKCRKPCS
 481 VTSYQLSAGY SRWPSVTSQE WVFQMLSRQN NYTVNNKRNG VAKVNIFFKE LNYKTNSESP
 541 SVTMVTLLSN LGSQWSLWFG SSVLSVVEMA ELVFDLLVIM FLMLLRRFRS RYWSPGRGGR
 601 GAQEVASTLA SSPPSHFCPH PMSLSLSQPG PAPSPALTAP PPAYATLGPR PSPGGSAGAS
 661 SSTCPLGGP
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Amiloride-sensitive sodium channel subunit gamma [Homo sapiens]
Epithelial Na(+) channel subunit gamma
Gamma-ENaC
649 aa protein
Accession: P51170.4 GI: 108885072

mRNA: SEQ ID NO:9

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  61 acccgcacga gccttggacc ctttggaacc gaaagcacgc ccgtcctcag agtcccgtcc
 121 tcaaagtccc atcctcgcca tggcacccgg agagaagatc aaagccaaaa tcaagaagaa
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 241 caacaccaac acccatggct gtcgccgcat cgtggtgtcc cgcggccgtc tgcgccgcct
 301 cctctggatc gggttcacac tgactgccgt ggccctcatc ctctggcagt gcgccctcct
 361 cgtcttctcc ttctatactg tctcagtttc catcaaagtc cacttccgga agctggattt
 421 tcctgcagtc accatctgca acatcaaccc ctacaagtac agcaccgttc gccaccttct
 481 agctgacttg gaacaggaga ccagagaggc cctgaagtcc ctgtatggct ttccagagtc
 541 ccggaagcgc cgaggaggcgg agtcctggaa ctccgtctca gagggaaagc agcctagatt
 601 ctcccaccgg attccgctgc tgatctttga tcaggatgag aagggcaagg ccagggactt
 661 cttcacaggg aggaagcgga aagtcggcgg tagcatcatt cacaaggctt caaatgtcat
 721 gcacatcgag tccaagcaag tggtgggatt ccaactgtgc tcaaatgaca cctccgactg
 781 tgccacctac accttcagct cgggaatcaa tgccattcag gagtggtata agctacacta
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2581 tttgtgaata aactgttctt catcattgac actggagaaa ggtgtcctcc atgccctcag
2641 gcagcagaga actggcccag agcccttgga gtgttggtgg agatcagagt gccgtggtgg
2701 aggtctggga ctatgtcaga gtgtcctcac tttggggcat gggtgggtcc aggagatgga
2761 tttagttatt caattttgtg gatgaataaa ttgaggcaca gaaagattaa gttaccggcc
2821 caaggtgaca cagtgaggag gtggcagagc taggatttga acccagacaa tctgacttca
2881 tgattttgca tccaattcgt gtctgtgcct tttaaagggt gaggtctgtg tcactttggg
2941 tggggagggg gagcatggtg ggccatgctc tgggcagctg ttccaagaca gagctgaccc
3001 ttccattacc aatggcctgt ccctcaccaa caagccaact gccacagatg acccacttca
3061 taccacattc acatctttcc acctgaaatg gctaacaggt attaaatcct tggttggtgt
3121 ttaaagccaa cccaagaaca gggtgttagg tactgtttta agcacctaga taggttagca
3181 taggggactg caaatggtgt tccgcaaagc aaatcctgtt tttgaccatg aactaagaat
3241 tttattttat tttatttttt atttttgta gaaataggat ctcactatgt tgcccaggct
3301 gttcttgaac tcctggcctc aaatgatctt cccacttcag cctcccaaag tgcagggatt
3361 acaggcacaa gccaccgtgc ccagccaaga atttttattt ttgcttttaa atagcaaaag
3421 aagaagattc tgtgactcat gaaaatgata tgagattcga attccagtgt ctataaataa
```

```
3481 agttttattg gtacacagca aaaaaaaaaa aaaaaa
```

Protein: SEQ ID NO:10

```
   1 MAPGEKIKAK IKKNLPVTGP QAPTIKELMR WYCLNTNTHG CRRIVVSRGR LRRLLWIGFT
  61 LTAVALILWQ CALLVFSFYT VSVSIKVHFR KLDFPAVTIC NINPYKYSTV RHLLADLEQE
 121 TREALKSLYG FPESRKRREA ESWNSVSEGK QPRFSHRIPL LIFDQDEKGK ARDFFTGRKR
 181 KVGGSIIHKA SNVMHIESKQ VVGFQLCSND TSDCATYTFS SGINAIQEWY KLHYMNIMAQ
 241 VPLEKKINMS YSAEELLVTC FFDGVSCDAR NFTLFHHPMH GNCYTFNNRE NETILSTSMG
 301 GSEYGLQVIL YINEEEYNPF LVSSTGAKVI IHRQDEYPFV EDVGTEIETA MVTSIGMHLT
 361 ESFKLSEPYS QCTEDGSDVP IRNIYNAAYS LQICLHSCFQ TKMVEKCGCA QYSQPLPPAA
 421 NYCNYQQHPN WMYCYYQLHR AFVQEELGCQ SVCKEACSFK EWTLTTSLAQ WPSVVSEKWL
 481 LPVLTWDQGR QVNKKLNKTD LAKLLIFYKD LNQRSIMESP ANSIEMLLSN FGGQLGLWMS
 541 CSVVCVIEII EVFFIDFFSI IARRQWQKAK EWWAWKQAPP CPEAPRSPQG QDNPALDIDD
 601 DLPTFNSALH LPPALGTQVP GTPPPKYNTL RLERAFSNQL TDTQMLDEL
```

Amiloride-sensitive sodium channel subunit delta [Homo sapiens]
Epithelial Na(+) channel subunit delta
Delta-ENaC
638 aa protein
Accession: P51172.2 GI: 116242784

mRNA: SEQ ID NO:11

```
   1 atggctgagc accgaagcat ggacgggaga atggaagcag ccacacgggg gggctctcac
  61 ctccaggctg cagcccagac gccccccagg ccggggccac catcagcacc accaccacca
 121 cccaaggagg ggcaccagga ggggctggtg gagctgcccg cctcgttccg ggagctgctc
 181 accttcttct gcaccaatgc caccatccac ggcgccatcc gcctggtctg ctcccgcggg
 241 aaccgcctca agacgacgtc ctgggggctg ctgtccctgg gagccctggt cgcgctctgc
 301 tggcagctgg ggctcctctt tgagcgtcac tggcaccgcc cggtcctcat ggccgtctct
 361 gtgcactcgg agcgcaagct gctcccgctg gtcaccctgt gtgacgggaa cccacgtcgg
 421 ccgagtccgg tcctccgcca tctggagctg ctggacgagt ttgccaggga gaacattgac
 481 tccctgtaca acgtcaacct cagcaaaggc agagccgccc tctccgccac tgtcccccgc
 541 cacgagcccc ccttccacct ggaccgggag atccgtctgc agaggctgag ccactcgggc
 601 agccgggtca gagtggggtt cagactgtgc aacagcacgg gcggcgactg cttttaccga
 661 ggctacacgt caggcgtggc ggctgtccag gactggtacc acttccacta tgtggatatc
 721 ctggccctgc tgcccgcggc atgggaggac agccacggga gccaggacgg ccacttcgtc
 781 ctctcctgca gttacgatgg cctggactgc caggcccgac agttccggac cttccaccac
 841 cccacctacg gcagctgcta cacggtcgat ggcgtctgga cagctcagcg ccccggcatc
 901 acccacggag tcggcctggt cctcagggtt gagcagcagc ctcacctccc tctgctgtcc
 961 acgctggccg gcatcagggt catggttcac ggccgtaacc acacgccctt cctggggcac
1021 cacagcttca gcgtccggcc agggacggag gccaccatca gcatccgaga ggacgaggtg
1081 caccggctcg ggagccccta cggccactgc accgccggcg gggaaggcgt ggaggtggag
1141 ctgctacaca acacctccta caccaggcag gcctgcctgg tgtcctgctt ccagcagctg
1201 atggtggaga cctgctcctg tggctactac ctccaccctc tgccggcggg ggctgagtac
1261 tgcagctctg cccggcaccc tgcctgggga cactgcttct accgcctcta ccaggacctg
1321 gagacccacc ggctcccctg tacctcccgc tgccccaggc cctgcaggga gtctgcattc
1381 aagctctcca ctgggacctc caggtggcct tccgccaagt cagctggatg gactctggcc
1441 acgctaggtg aacaggggct gccgcatcag agccacagac agaggagcag cctggccaaa
1501 atcaacatcg tctaccagga gctcaactac cgctcagtgg aggaggcgcc cgtgtactcg
1561 gtgccgcagc tgctctcggc catgggcagc ctctgcagcc tgtggtttgg ggcctccgtc
1621 ctctccctcc tggagctcct ggagctgctg ctcgatgctt ctgccctcac cctggtgcta
1681 ggcggccgcc ggctccgcag ggcgtggttc tcctggccca gagccagccc tgcctcaggg
1741 gcgtccagca tcaagccaga ggccagtcag atgcccccgc ctgcaggcgg cacgtcagat
1801 gacccggagc ccagcgggcc tcatctccca cgggtgatgc ttccaggggt tctggcggga
1861 gtctcagccg aagagagctg ggctgggccc cagcccctg agactctgga cacctga
```

Protein: SEQ ID NO:12

```
   1 MAEHRSMDGR MEAATRGGSH LQAAAQTPPR PGPPSAPPPP PKEGHQEGLV ELPASFRELL
  61 TFFCTNATIH GAIRLVCSRG NRLKTTSWGL LSLGALVALC WQLGLLFERH WHRPVLMAVS
 121 VHSERKLLPL VTLCDGNPRR PSPVLRHLEL LDEFARENID SLYNVNLSKG RAALSATVPR
 181 HEPPFHLDRE IRLQRLSHSG SRVRVGFRLC NSTGGDCFYR GYTSGVAAVQ DWYHFHYVDI
 241 LALLPAAWED SHGSQDGHFV LSCSYDGLDC QARQFRTFHH PTYGSCYTVD GVWTAQRPGI
 301 THGVGLVLRV EQQPHLPLLS TLAGIRVMVH GRNHTPFLGH HSFSVRPGTE ATISIREDEV
 361 HRLGSPYGHC TAGGEGVEVE LLHNTSYTRQ ACLVSCFQQL MVETCSCGYY LHPLPAGAEY
 421 CSSARHPAWG HCFYRLYQDL ETHRLPCTSR CPRPCRESAF KLSTGTSRWP SAKSAGWTLA
 481 TLGEQGLPHQ SHRQRSSLAK INIVYQELNY RSVEEAPVYS VPQLLSAMGS LCSLWFGASV
 541 LSLLELLELL LDASALTLVL GGRRLRRAWF SWPRASPASG ASSIKPEASQ MPPPAGGTSD
 601 DPEPSGPHLP RVMLPGVLAG VSAEESWAGP QPLETLDT
```

* * * * *

[0121] For all purposes in the United States of America, each and every publication and patent document cited herein is incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

[0122] While the invention has been described with reference to the specific embodiments, changes can be made and equivalents can be substituted to adapt to a particular context or intended use, thereby achieving benefits of the invention without departing from the scope of what is claimed.

CLAIMS

The invention claimed is:

1. A method of birth control, contragestion, inducing labor, or inhibiting COX-2 in the uterus of a subject, comprising administering to the subject an effective dose of an inhibitor of an endometrial epithelium sodium channel.

2. The method of claim 1, wherein the inhibitor is a small molecule drug selected from amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET).

3. The method of claim 1, wherein the inhibitor is an antisense RNA, interfering RNA, or siRNA that inhibits expression of said endometrial epithelium sodium channel.

4. The method of claim 1, wherein the inhibitor is an antibody that specifically binds and thereby blocks said endometrial epithelium sodium channel.

5. The method of any of claims 1 to 4, which is a method of birth control.

6. The method of aany of claims 1 to 4, which is a method for inducing labor.

7. The method of any of claims 1 to 6, which is a method for inhibiting uterine COX-2.

8. The method of any of claims 1 to 7, wherein the inhibitor is administered into the uterus of the subject.

9. A method of treating an inflammatory condition in a subject, comprising administering to the subject an effective dose of an inhibitor of an epithelium sodium channel at or near the site of inflammation.

10. The method of claim 9, wherein the inhibitor is a small molecule drug selected from amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET).

11. The method of claim 9, wherein the inhibitor is an antisense RNA, interfering RNA, or siRNA that inhibits expression of said epithelium sodium channel.

12. The method of claim 9, wherein the inhibitor is an antibody that specifically binds and thereby blocks said epithelium sodium channel.

13. The method of any of claims 9 to 12, which is a method for inhibiting COX-2.

14. The method of any of claims 9 to 13, wherein the inhibitor is administered at or around the site of the inflammation.

15. A method for improving the probability of implantation in a subject of an *in vitro* fertilized (IVF) embryo, comprising increasing the expression or activity of an endometrial epithelium sodium channel (ENaC) in the subject.

16. The method of claim 15, wherein the increasing the expression or activity of ENaC comprises administering into the uterus of a subject an effective amount of a serine protease.

17. A method of identifying a pharmaceutical product as an agent for birth control or contragestion, comprising:

a) obtaining a compound that reduces expression of an endometrial epithelium channel and/or inhibits transport of sodium ion through said channel;

b) administering said compound to a test subject at or around the time when an embryo is present in the uterus of the subject following fertilization; and then

c) determining whether the compound affects implantation of said embryo in the uterus of the subject.

18. A method for producing a pharmaceutical composition for birth control, the method comprising obtaining a compound that was identified according to the method of claim 17, and then formulating an effective amount of the compound with a pharmaceutically compatible excipient for administration to the uterus of a human subject.

19. A pharmaceutical composition formulated and labeled for use in birth control in a human subject, comprising an effective amount of a compound that inhibits an endometrial epithelium sodium channel.

20. The pharmaceutical composition of claim 19, wherein the compound is a small molecule drug selected from amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET).

21. The pharmaceutical composition of claim 19, wherein the compound is an antisense RNA, interfering RNA, or siRNA that inhibits expression of said endometrial epithelium sodium channel.

22. The pharmaceutical composition of claim 19, wherein the compound is an antibody that specifically binds and thereby blocks said endometrial epithelium sodium channel.

23. A method of identifying a pharmaceutical product as an agent for treating an inflammatory condition, comprising:

a) obtaining a compound that reduces expression of an epithelium channel and/or inhibits transport of sodium ion through said channel;

b) administering said compound to a test subject who has an inflammatory condition; and then

c) determining whether the compound reduces inflammation in the subject.

24. A method for producing a product for treating an inflammatory condition, comprising obtaining a compound that was identified according to the method of claim 23, and then formulating an effective amount of the compound with a pharmaceutically compatible excipient for administration to the uterus of a human subject.

25. A pharmaceutical composition formulated and labeled for use in treating an inflammatory condition in a human subject, comprising an effective amount of a compound that inhibits an epithelium sodium channel.

26. The pharmaceutical composition of claim 25, wherein the compound is a small molecule drug selected from amiloride, Ac-LHPHLQRL-amide, and an epoxyeicosatrienoic acid (EET).

27. The pharmaceutical composition of claim 25, wherein the compound is an antisense RNA, interfering RNA, or siRNA that inhibits expression of said epithelium sodium channel.

28. The pharmaceutical composition of claim 25, wherein the compound is an antibody that specifically binds and thereby blocks said epithelium sodium channel.

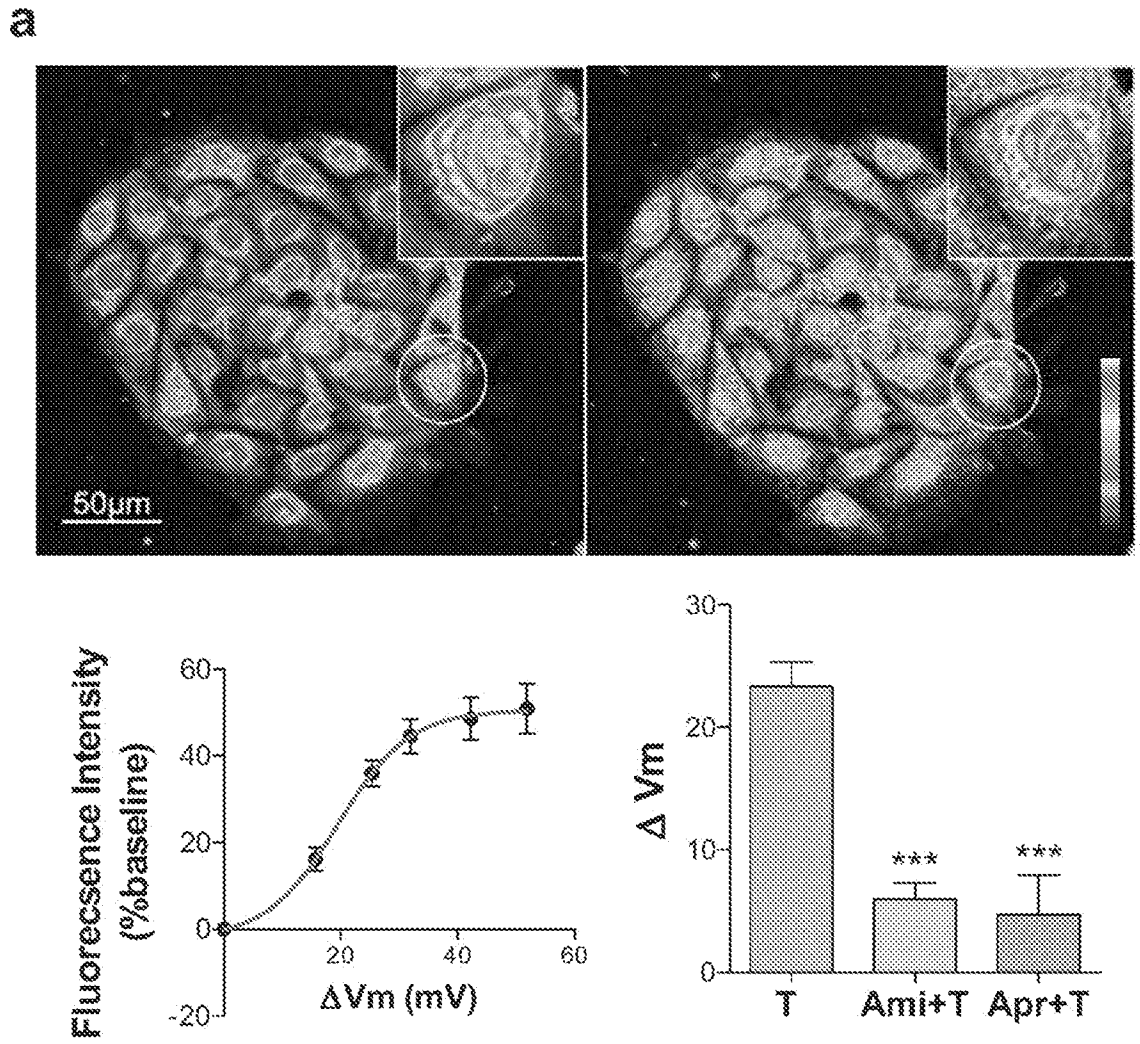

a
50μm
Fluorescence Intensity (%baseline)
60
40
20
0
-20
20
40
60
ΔVm (mV)
Δ Vm
30
20
10
0
***
***
T
Ami+T
Apr+T

FIG. 1

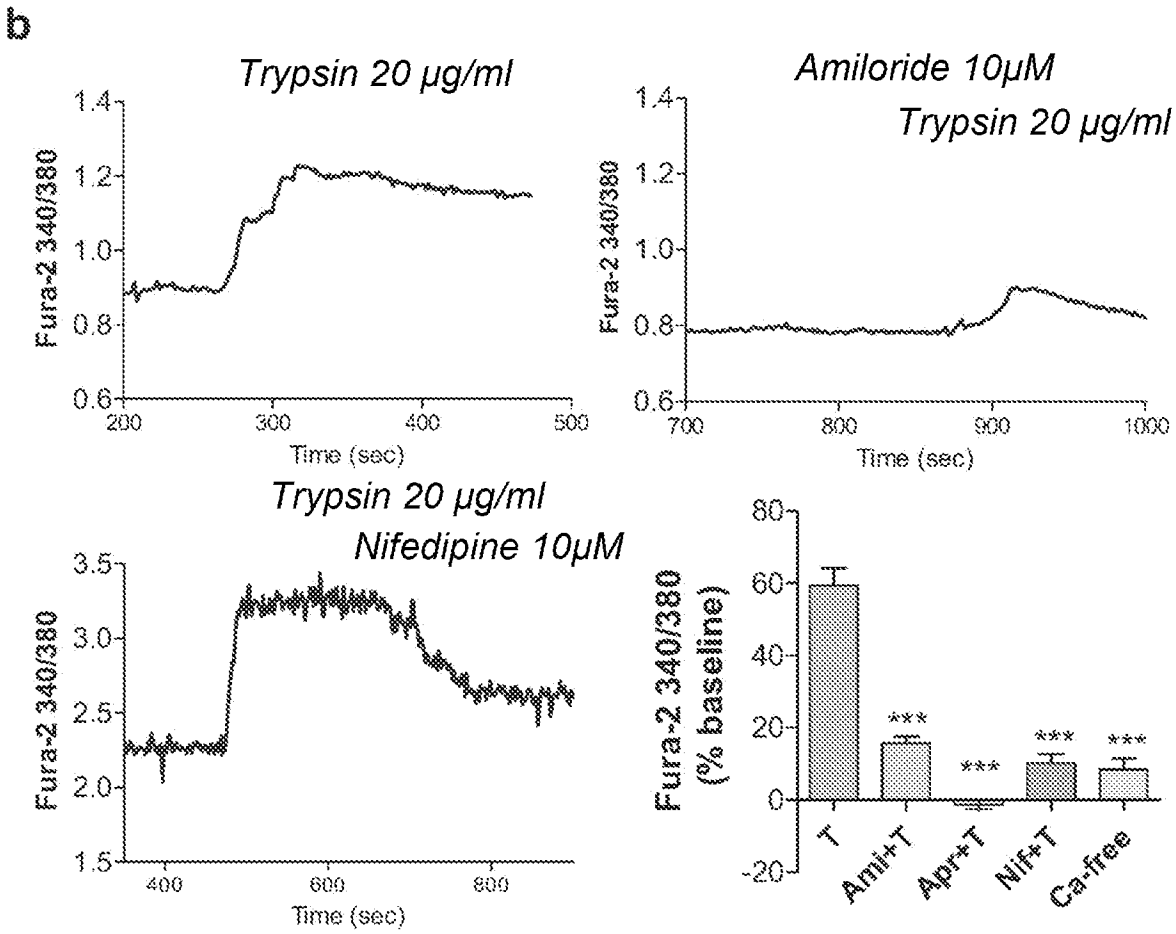

b
Trypsin 20 µg/ml
Fura-2 340/380
Time (sec)
Amiloride 10µM
Trypsin 20 µg/ml
Fura-2 340/380
Time (sec)
Trypsin 20 µg/ml
Nifedipine 10µM
Fura-2 340/380
Time (sec)
Fura-2 340/380 (% baseline)
T
Ami+T
Apr+T
Nif+T
Ca-free

FIG. 1 (cont'd)

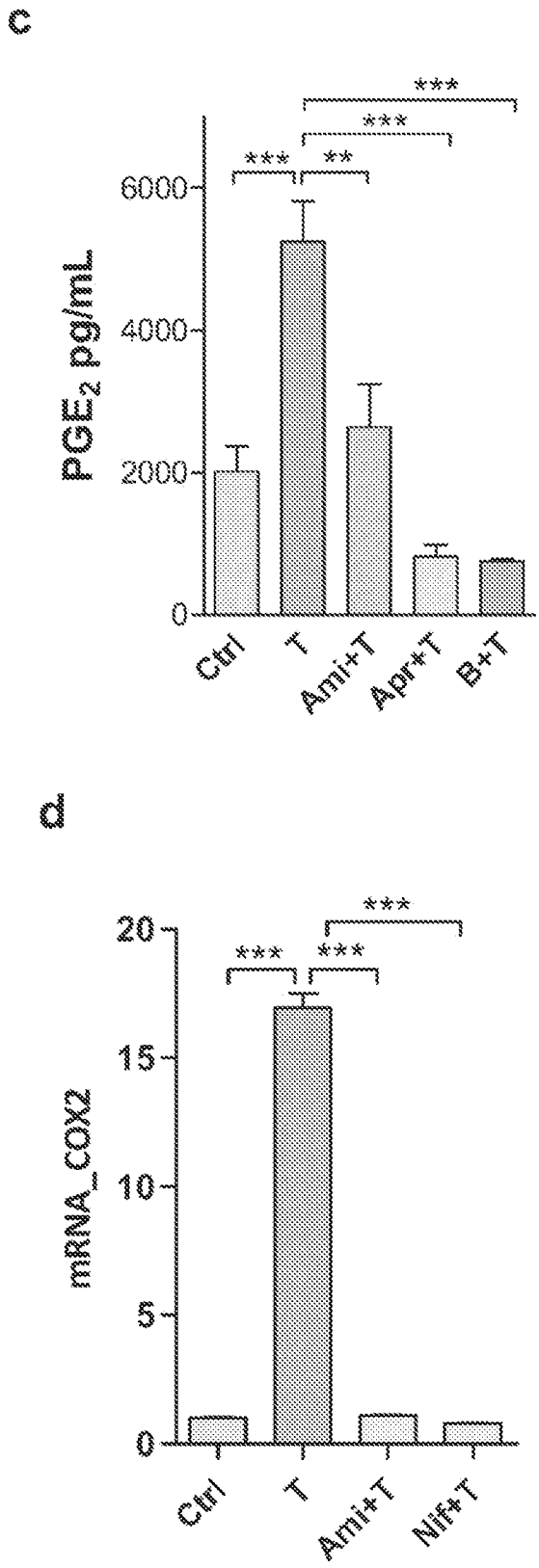

c
***
***
***
**
6000
4000
2000
0
PGE2 pg/mL
Ctrl
T
Ami+T
Apr+T
B+T
d
***
***
***
20
15
10
5
0
mRNA_COX2
Ctrl
T
Ami+T
Nif+T

FIG. 1 (cont'd)

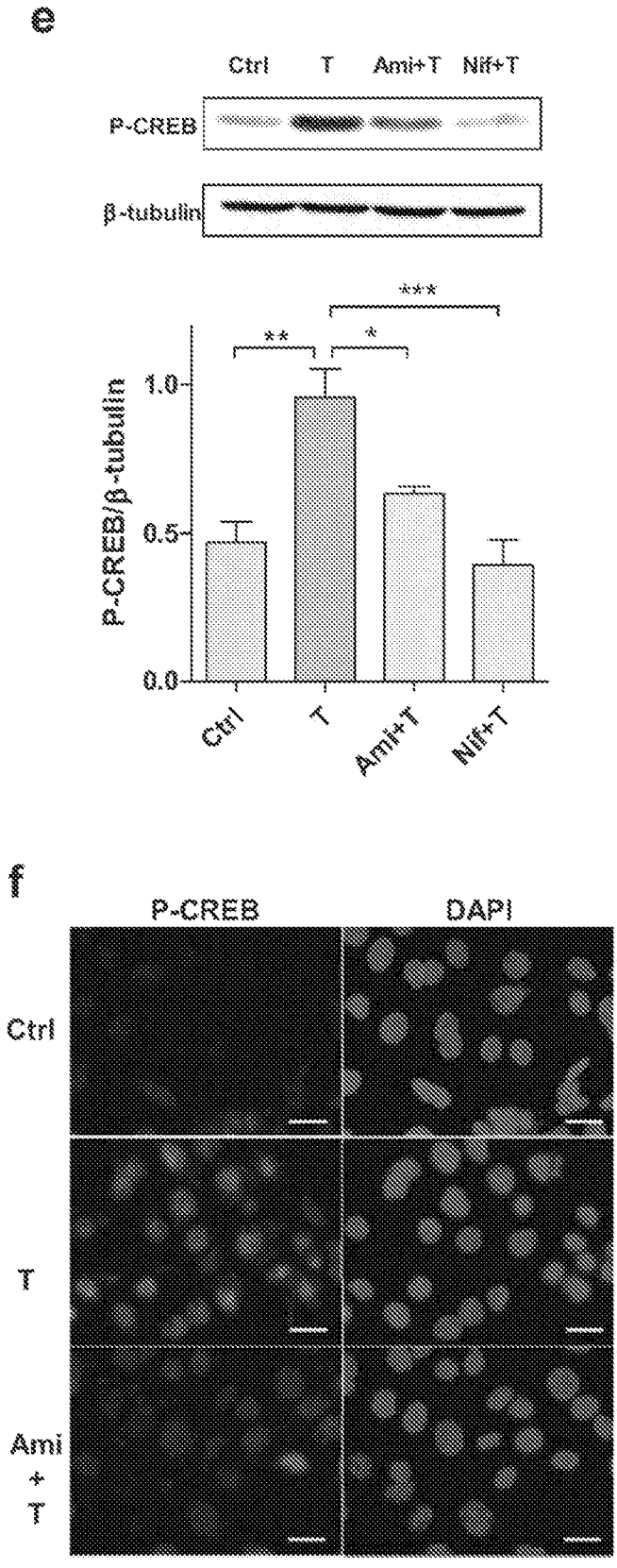

e
Ctrl
T
Ami+T
Nif+T
P-CREB
β-tubulin
***
**
*
P-CREB/β-tubulin
1.0
0.5
0.0
Ctrl
T
Ami+T
Nif+T
f
P-CREB
DAPI
Ctrl
T
Ami
+
T

FIG. 1 (cont'd)

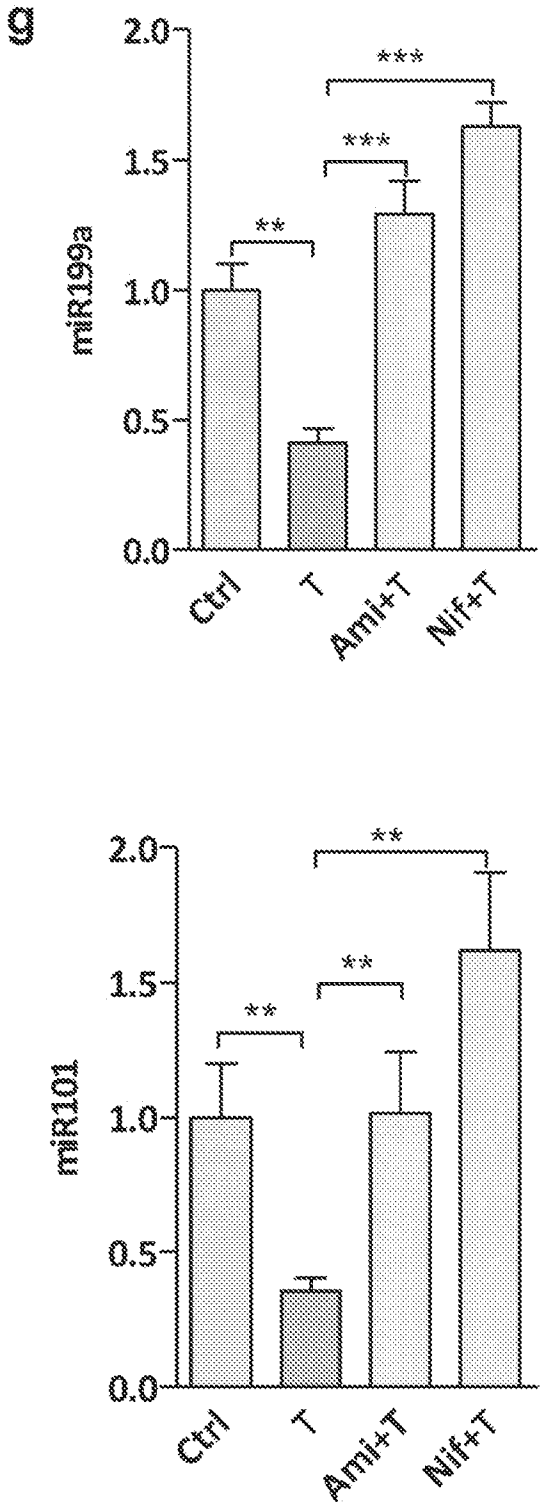

g
miR199a
2.0
1.5
1.0
0.5
0.0
**
***
***
Ctrl
T
Ami+T
Nif+T
miR101
2.0
1.5
1.0
0.5
0.0
**
**
**
Ctrl
T
Ami+T
Nif+T

FIG. 1 (cont'd)

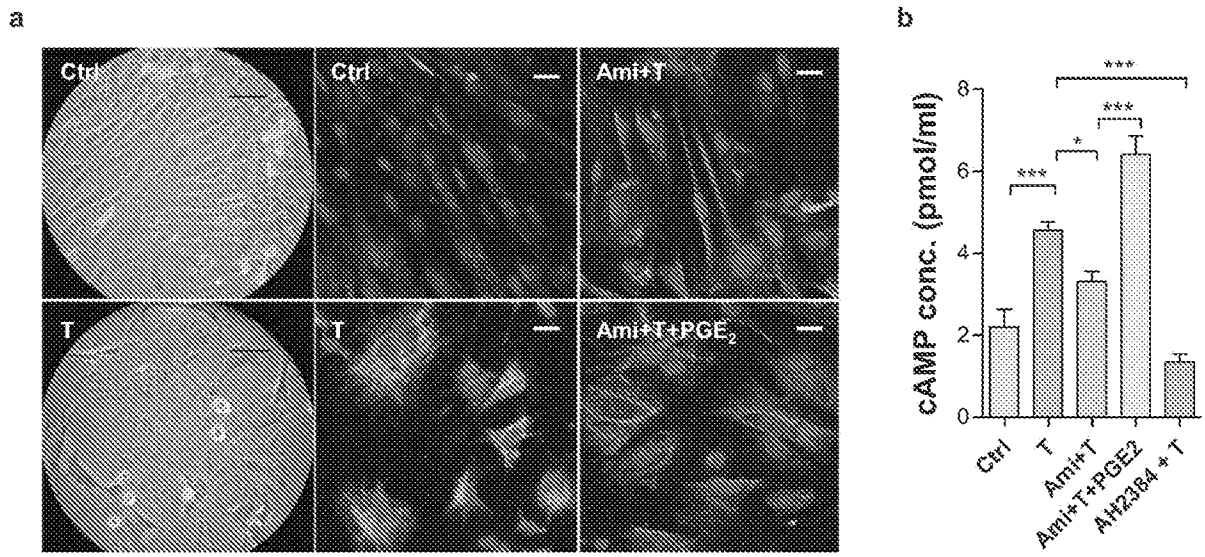

a
Ctrl
Ctrl
Ami+T
T
T
Ami+T+PGE2
b
cAMP conc. (pmol/ml)
0
2
4
6
8
***
**
*
***
***
Ctrl
T
Ami+T
Ami+T+PGE2
AH2364 + T

FIG. 2

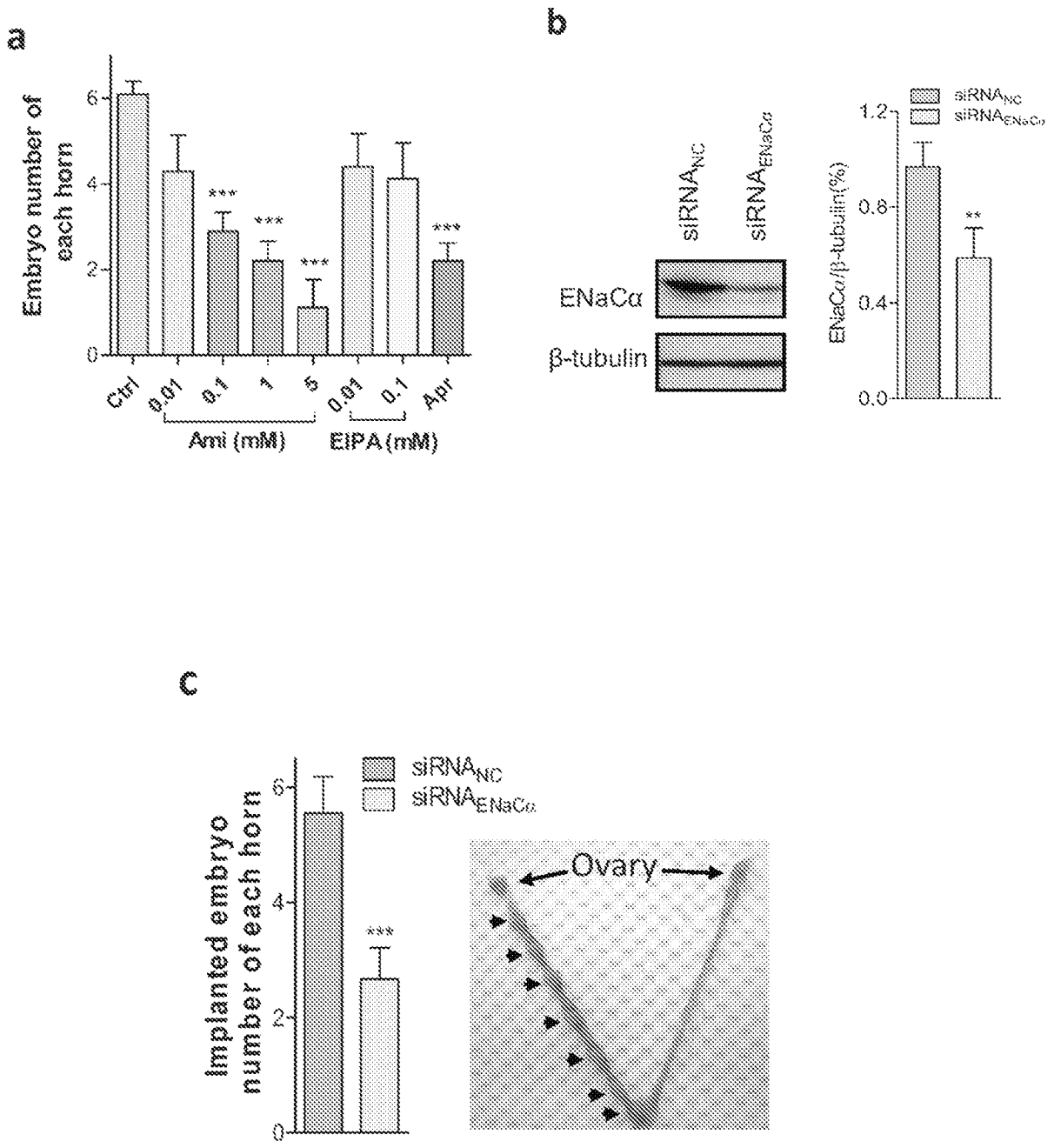

a
Embryo number of each horn
Ctrl
0.01
0.1
1
5
0.01
0.1
Apr
Ami (mM)
EIPA (mM)
b
siRNA$_{NC}$
siRNA$_{ENaCα}$
ENaCα
β-tubulin
ENaCα/β-tubulin(%)
c
Implanted embryo number of each horn
Ovary

FIG. 3

d

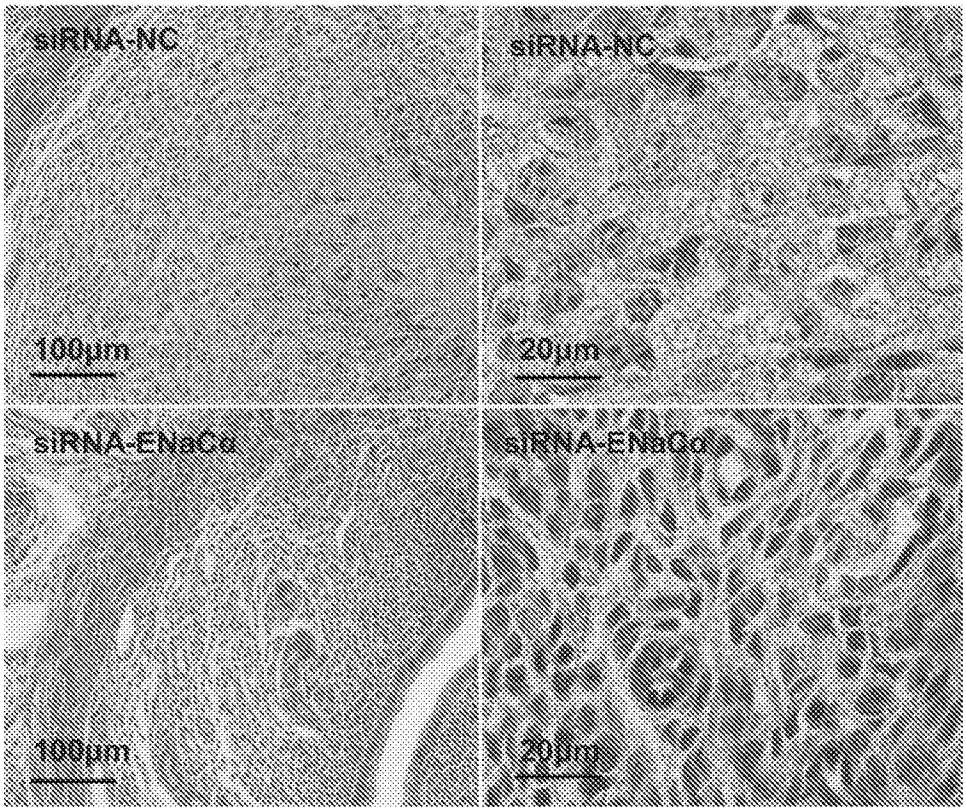

siRNA-NC
100μm
siRNA-NC
20μm
siRNA-ENaCα
100μm
siRNA-ENaCα
20μm

FIG. 3 (cont'd)

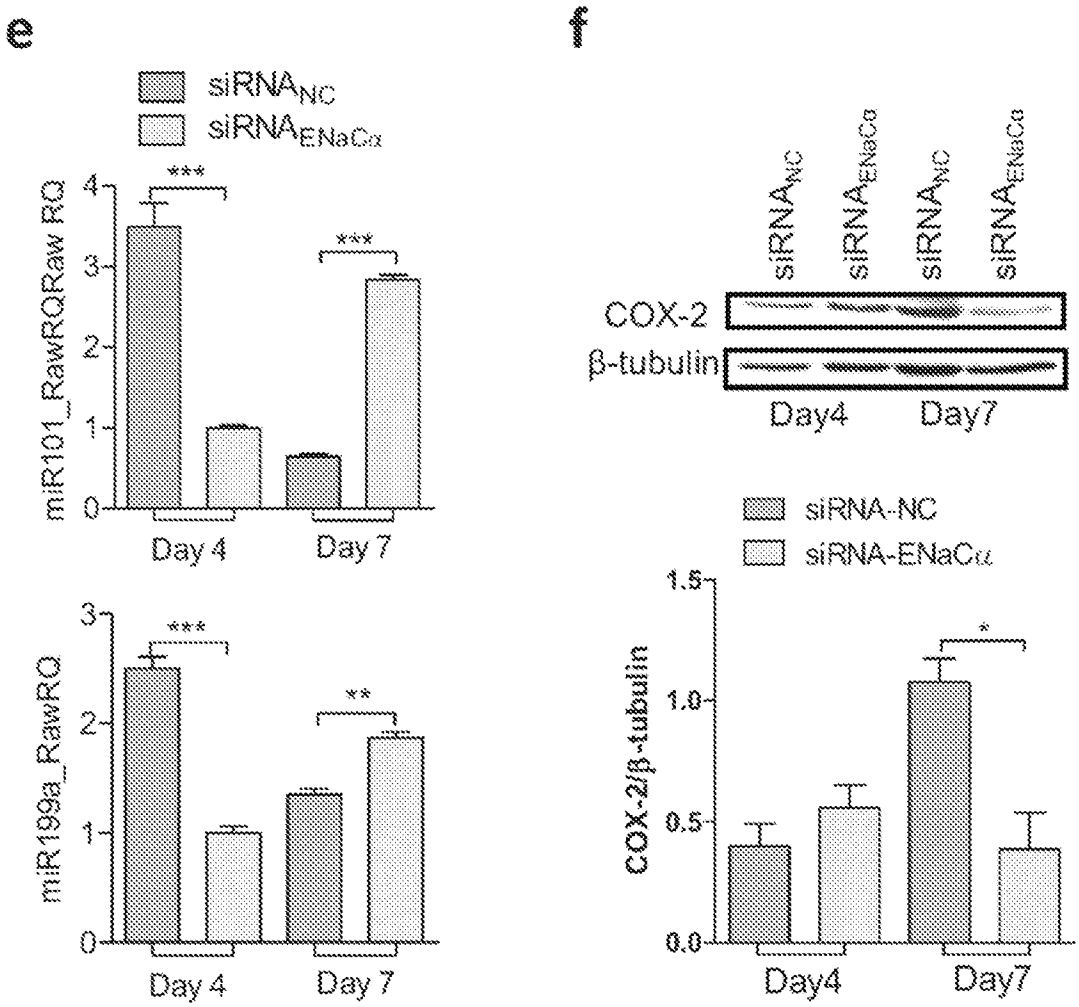
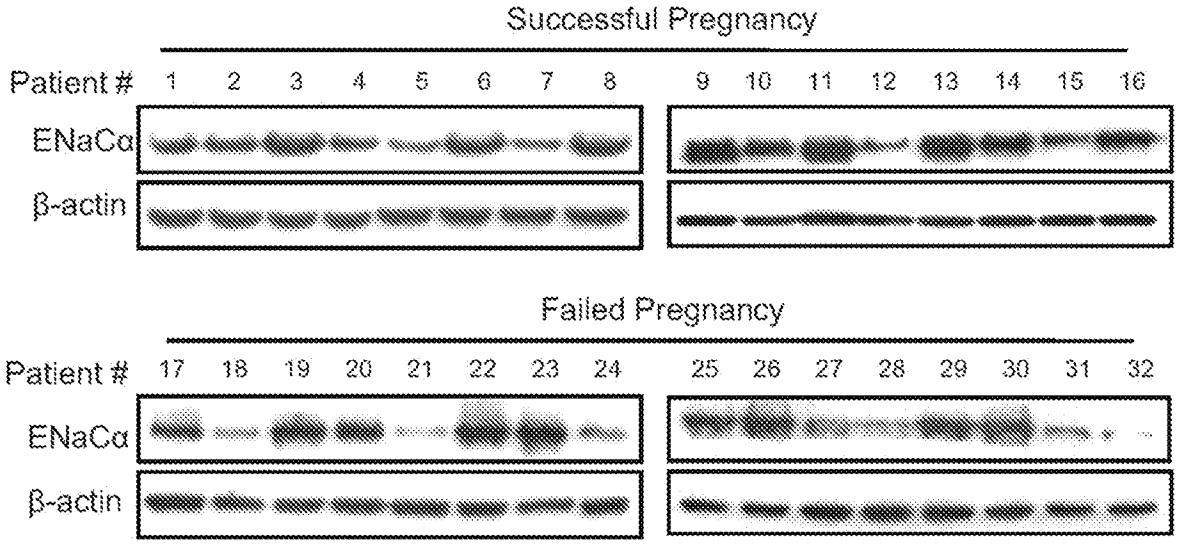

e
siRNA$_{NC}$
siRNA$_{ENaCα}$
miR101_RawRQRaw RQ
***
***
Day 4
Day 7
miR199a_RawRQ
***
**
Day 4
Day 7
f
siRNA$_{NC}$
siRNA$_{ENaCα}$
siRNA$_{NC}$
siRNA$_{ENaCα}$
COX-2
β-tubulin
Day4
Day7
siRNA-NC
siRNA-ENaCα
COX-2/β-tubulin
*
Day4
Day7

FIG. 3 (cont'd)


Successful Pregnancy
Patient # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
ENaCα
β-actin
Failed Pregnancy
Patient # 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
ENaCα
β-actin

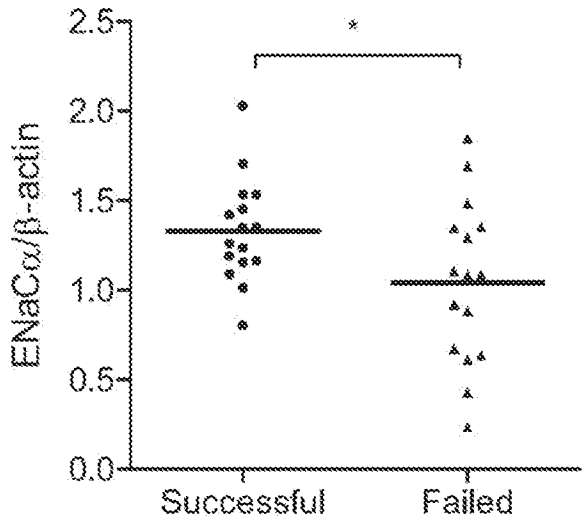

ENaCα/β-actin
2.5
2.0
1.5
1.0
0.5
0.0
*
Successful
Failed

FIG. 4

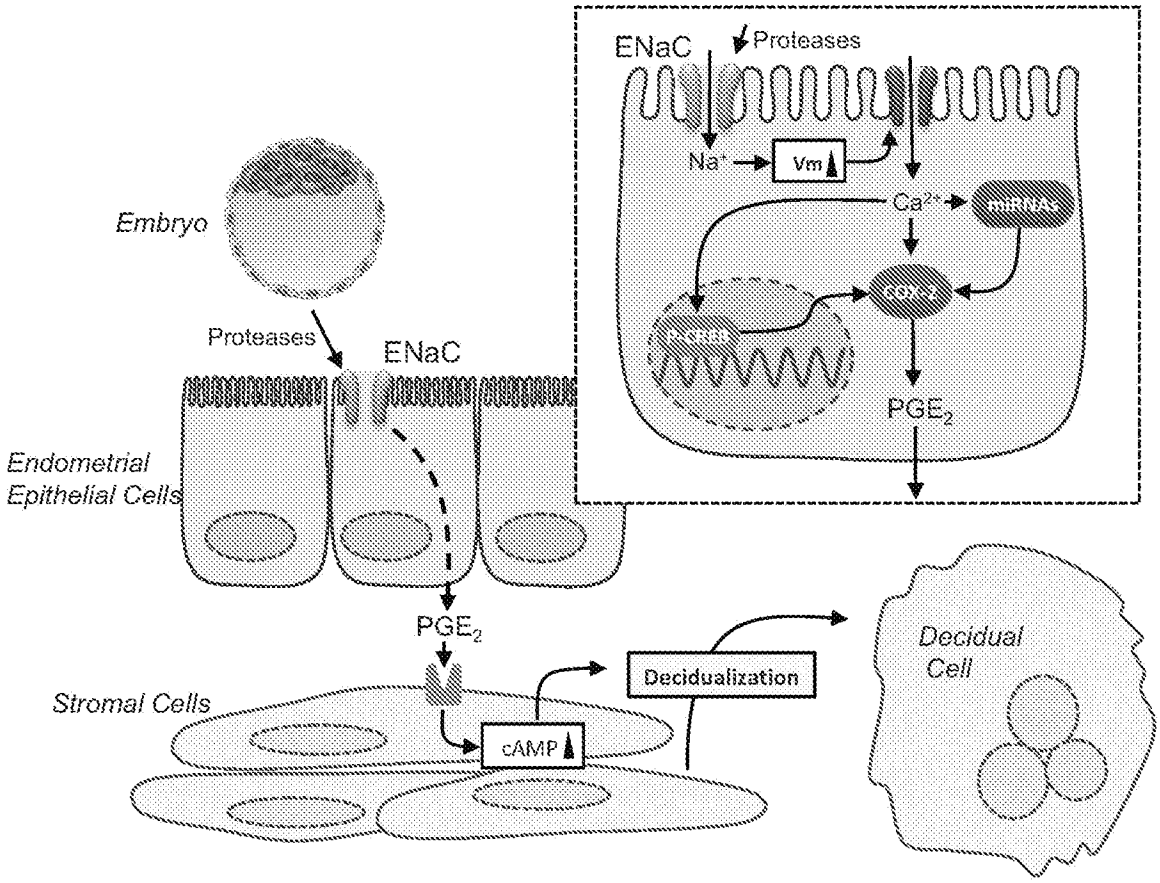
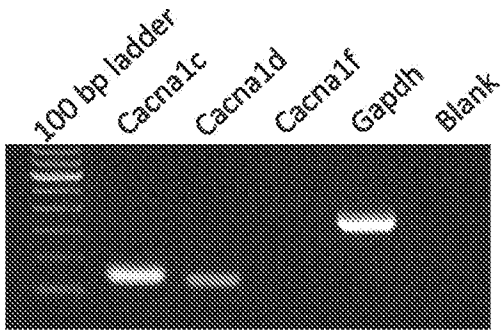

ENaC
Proteases
Na+
Vm
Ca2+
mRNA
COX-2
Protein
PGE2
Embryo
Proteases
ENaC
Endometrial
Epithelial Cells
PGE2
Stromal Cells
cAMP
Decidualization
Decidual
Cell

FIG. 5

a.


100 bp ladder
Cacna1c
Cacna1d
Cacna1f
Gapdh
Blank

b.

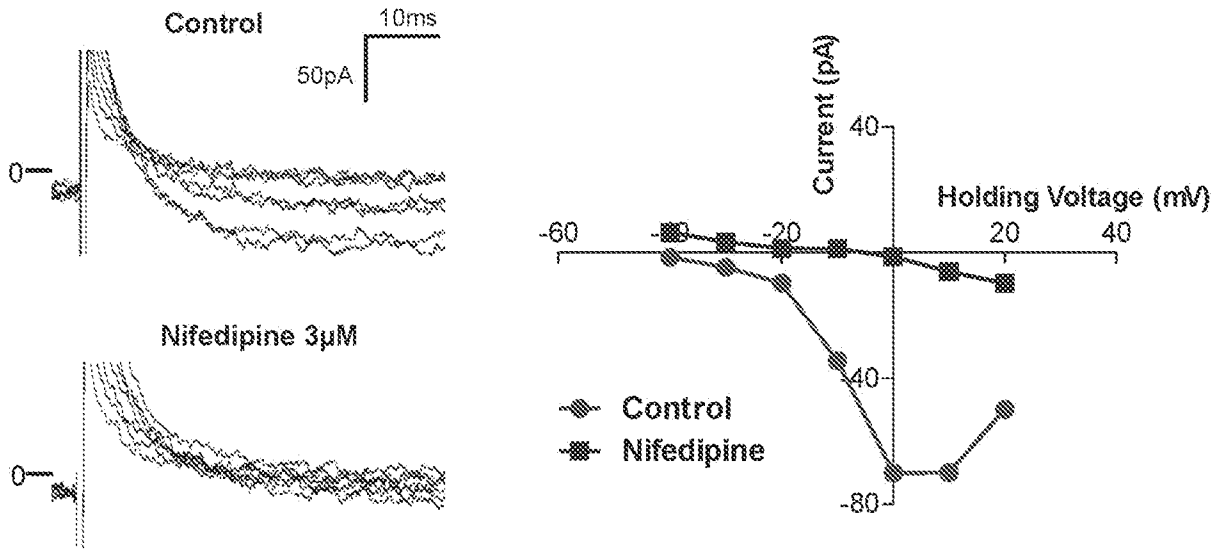
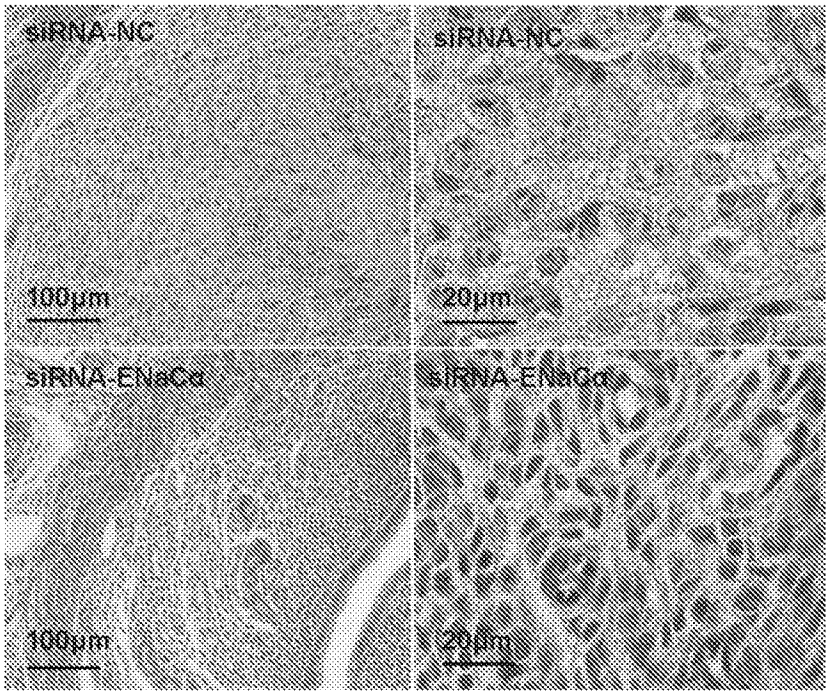

Control
10ms
50pA
0
Nifedipine 3μM
0
Current (pA)
40
-40
-80
-60
-20
20
40
Holding Voltage (mV)
Control
Nifedipine

FIG. 6

a.


siRNA-NC
siRNA-NC
100μm
20μm
siRNA-ENaCα
siRNA-ENaCα
100μm
20μm

b.

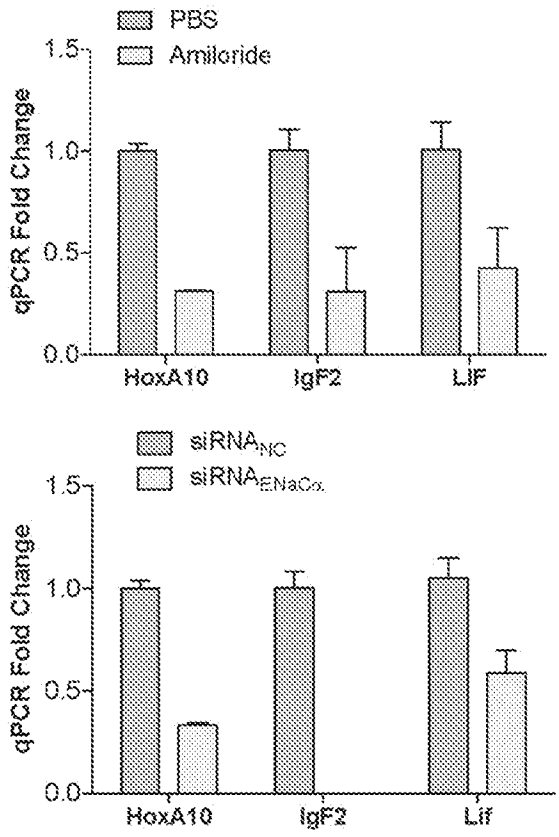

PBS
Amiloride
qPCR Fold Change
1.5
1.0
0.5
0.0
HoxA10
IgF2
LIF
siRNA$_{NC}$
siRNA$_{ENaCα}$
qPCR Fold Change
1.5
1.0
0.5
0.0
HoxA10
IgF2
Lif

FIG. 7

	Successful Pregnancy	Failed Pregnancy	P
Age (year)	30.69 ± 0.75	29.5 ± 0.63	NS
Length of menstruation cycle	28.94 ± 0.32	29.69 ± 0.37	NS
Number of oocytes	11.94 ± 1.31	11.69 ± 1.26	NS
Fertilization rate (%)	72.49 ± 2.43	73.21 ± 3.22	NS
Good quality embryo (%)	61.31 ± 4.35	60.00 ± 4.76	NS
Number of transferred embryo	2.06 ± 0.11	2 ± 0.09	NS
E2 (pmol/L)	8086.44 ± 1009	8878.69 ± 1110.63	NS
P4 (nmol/L)	1.85 ± 0.23	1.89 ± 0.15	NS
Thickness of endometrium (mm)	5.53 ± 0.29	5.05 ± 0.12	NS

	Successful Pregnancy	Failed Pregnancy	P
Age (year)	29.88 ± 1.26	29.71 ± 1.15	NS
Length of menstruation cycle	30.13 ± 0.80	29.94 ± 0.82	NS
Number of oocytes	12.00 ± 1.62	11.12 ± 1.35	NS
Fertilization rate (%)	89.75 ± 2.58	80.00 ± 5.65	NS
Good quality embryo (%)	75.06 ± 6.25	77.94 ± 5.47	NS
Number of transferred embryo	2.06 ± 0.14	1.82 ± 0.13	NS
E_2 (pmol/L)	11180 ± 1723	12360 ± 1584	NS
P_4 (pmol/L)	2.87 ± 0.43	2.48± 0.35	NS
Thickness of endometrium(mm)	11.55 ± 0.68	11.88 ± 0.41	NS

FIG. 8

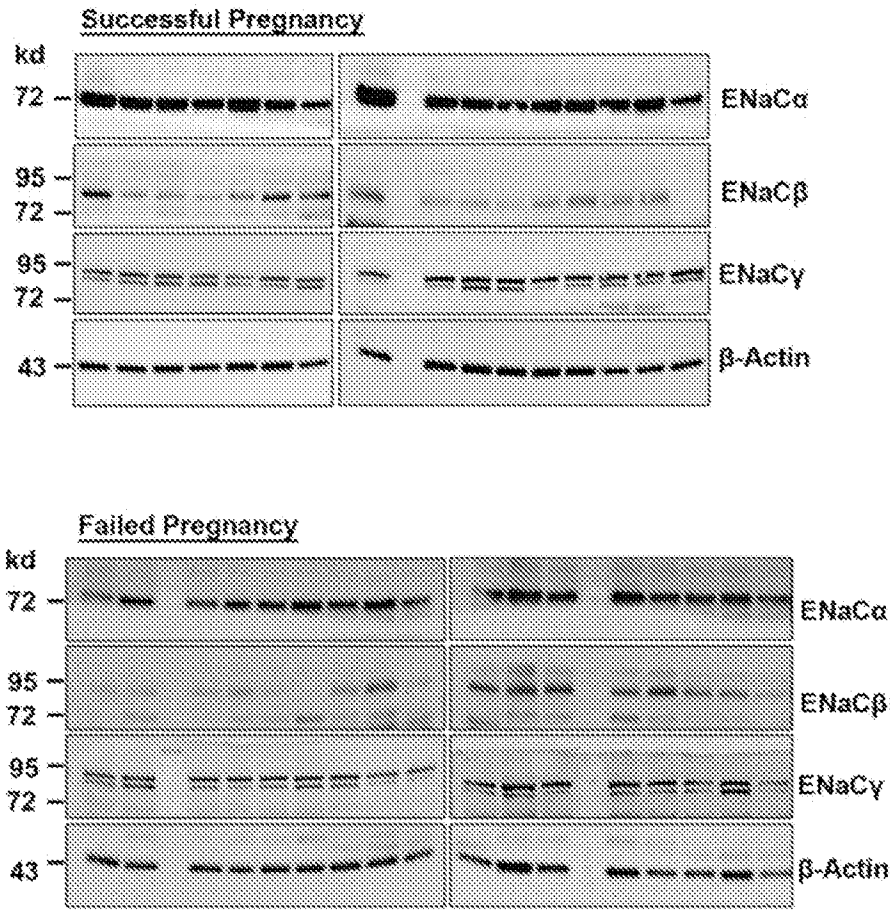

Successful Pregnancy
kd
72
95
72
95
72
43
ENaCα
ENaCβ
ENaCγ
β-Actin
Failed Pregnancy
kd
72
95
72
95
72
43
ENaCα
ENaCβ
ENaCγ
β-Actin

FIG. 9

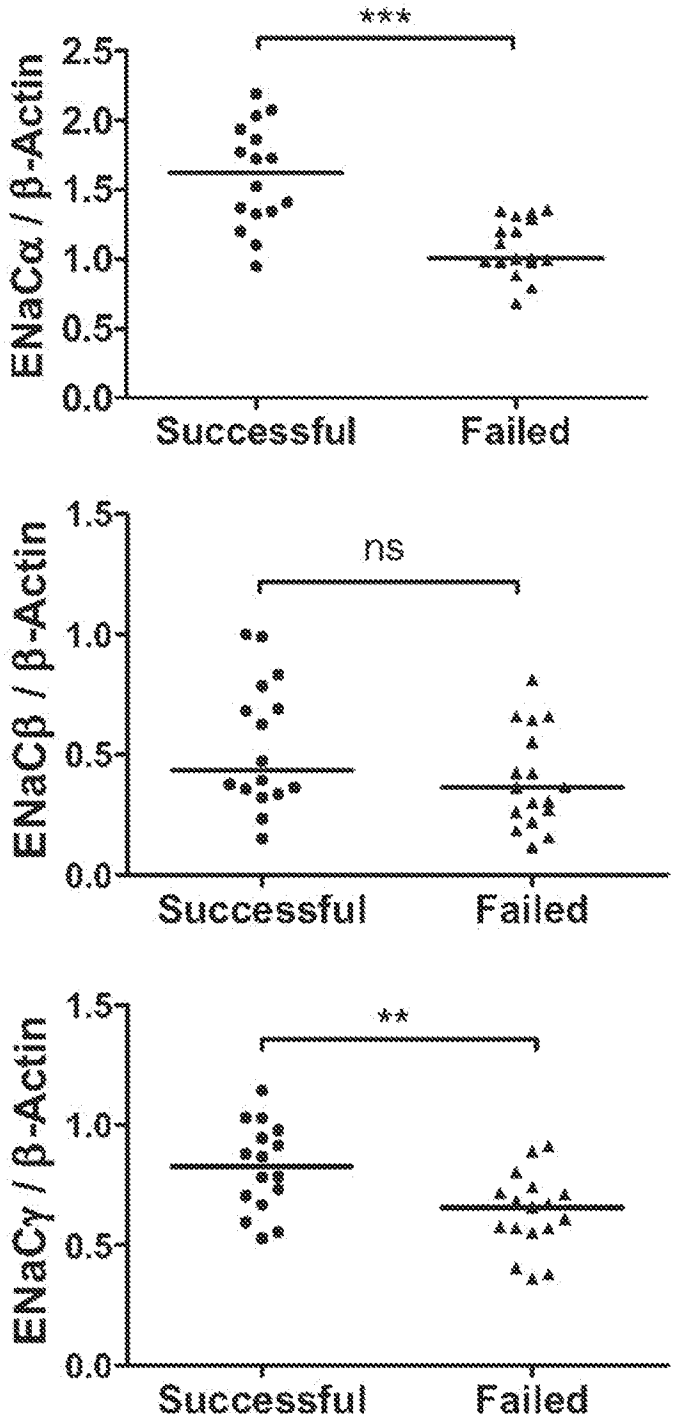

***
ENaCα / β-Actin
2.5
2.0
1.5
1.0
0.5
0.0
Successful
Failed
ns
ENaCβ / β-Actin
1.5
1.0
0.5
0.0
Successful
Failed
**
ENaCγ / β-Actin
1.5
1.0
0.5
0.0
Successful
Failed

FIG. 9 (cont'd)

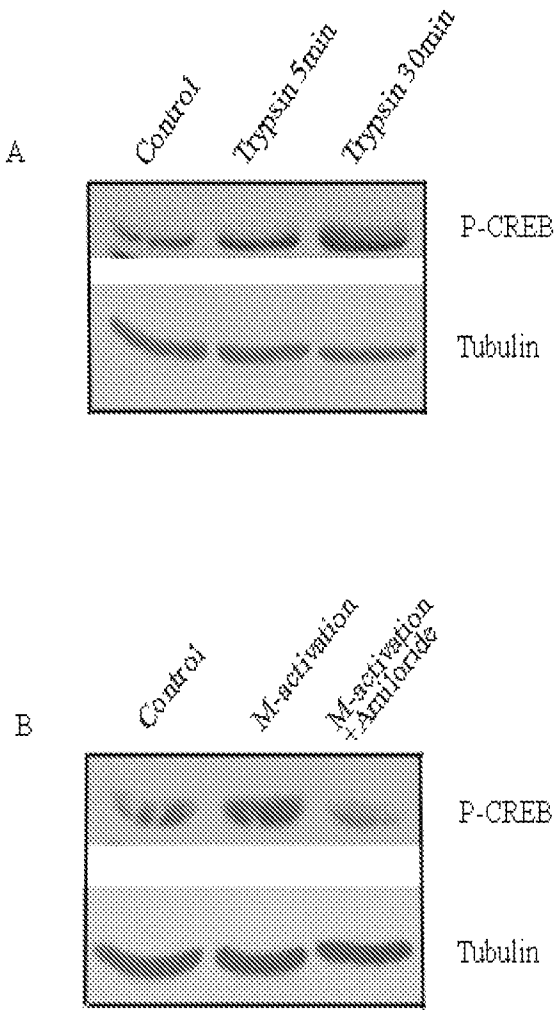

A
Control
Trypsin 5min
Trypsin 30min
P-CREB
Tubulin
B
Control
M-activation
M-activation +Amiloride
P-CREB
Tubulin

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/036358

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4965 (2006.01) A61K 38/08 (2006.01) A61K 31/185 (2006.01) A61K 39/395 (2006.01) C12N 15/113 (2010.01) A61P 15/04 (2006.01) A61P 15/08 (2006.01) A61P 15/18 (2006.01) A61P 29/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, WPIDS, EPODOC. Keywords: Epitheli+ sodium channel, ENaC, sodium channel non-voltage gated-1, SCNN1?, amiloride sensitive sodium channel, birth control, contragestion, induc+ lab[ou,o]r, fertili+, implant+, endometri+, uteri+, uterus, +inflamma+, COX-2, cyclooxygenase-2, contract+, +amiloride, Ac-LHPHLQRL-amide, epoxyeicosatrienoic acid, EET, triamterene, benzamil, siRNA, antisense, interfere+ RNA, miRNA, antibod+, immunoglob+, and similar terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2013	06 August 2013

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INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/US2013/036358
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU, Z. et al. "Preventive but not late amiloride therapy reduces morbidity and mortality of lung disease in betaENaC-overexpressing mice." Am J Respir Crit Care Med. 2008 Dec 15; 178(12):1245-56. Epub 2008 Oct 10. (see abstract, page 1246 right column paragraph 2)	19, 20, 25 and 26
X	WO 2003/057847 A2 (ALGOS THERAPEUTICS, INC) 17 July 2003 (see page 9 last paragraph, page 16 paragraph 2 – page 19 last paragraph, Examples 1 and 2, claims)	19, 21, 25 and 27
X	WO 2010/062817 A1 (MERCK SHARP & DOHME CORP.) 03 June 2010 (see abstract, paragraph [0015], [0252]-[0253])	9, 11, 14, 19, 21, 25 and 27
X	WO 2011/044167 A1 (UNIVERSITY OF CALIFORNIA, SAN FRANSISCO & NAPO PHARMACEUTICALS INC.) 14 April 2011 (see paragraph [0033], [0102], Examples and Claims 5 and 29)	19 and 25
X	WO 2007/071369 A2 (NOVARTIS PHARMA GMBH) 28 June 2007 (see page 8 paragraphs 1 and 2, page 16 paragraph 1, page 17 paragraphs 4-6, page 20 last paragraph and claims 7-11)	9, 14, 19 and 25
X	WO 2009/074575 A2 (NOVARTIS AG) 18 June 2009 (see abstract, page 50 paragraph 2 and 3, page 54 last paragraph-page 57 paragraph 2, page 60 paragraph 2 and claims 10-14)	9, 14, 19 and 25
X	VALLET, V., et al. "An epithelial serine protease activates the amiloride-sensitive sodium channel." Nature. 1997 Oct 9; 389(6651):607-10. (see whole document)	19, 20, 25 and 26
X	KASHLAN, O.B. "Allosteric inhibition of the epithelial Na+ channel through peptide binding at peripheral finger and thumb domains." J Biol Chem. 05 November 2010. Epub 03 September 2010; 285(45):35216-23. (see whole document)	19 and 25
X	SUN, P., et al. "High potassium intake enhances the inhibitory effect of 11,12-EET on ENaC." J Am Soc Nephrol. 2010 Oct; 21(10):1667-77. Epub 2010 Jul 1. (see abstract)	19, 20, 25 and 26
X	PAVLOV, TS. et al. "Effects of cytochrome P-450 metabolites of arachidonic acid on the epithelial sodium channel (ENaC)." Am J Physiol Renal Physiol. 2011 Sep; 301(3):F672-81. Epub 2011 Jun 22. (see abstract)	19, 20, 25 and 26
X	BRIDGES, RJ., (& POLL, CT.) et al. "Na+ transport in normal and CF human bronchial epithelial cells is inhibited by BAY 39-9437." Am J Physiol Lung Cell Mol Physiol. 2001 Jul; 281(1):L16-23. (see abstract)	19 and 25
P,X	RUAN, YC., et al. "Activation of the epithelial Na+ channel triggers prostaglandin E_2 release and production required for embryo implantation." Nat Med. 2012 Jul;18(7):1112-1117. (see page 1116 both columns)	1-3, 5, 7, 8, 15, 17-21 and 25-27
	ENUKA, Y., et al. "Epithelial sodium channels (ENaC) are uniformly distributed on motile cilia in the oviduct and the respiratory airways." Histochem Cell Biol. 2012 Mar;137(3):339-53. Epub 2011 Dec 30.	

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2013/036358**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A		
A	CHAN, HC., et al. "Epithelial ion channels in the regulation of female reproductive tract fluid microenvironment: implications in fertility and infertility." Sheng Li Xue Bao. 2007 Aug 25; 59(4):495-504. (see whole document)	1-8, 15-22
A	KLEYMAN, TR., et al. "ENaC at the cutting edge: regulation of epithelial sodium channels by proteases." J Biol Chem. 2009 Jul 31; 284(31):20447-51. Epub 2009 Apr 28. (see whole document)	15 and 16

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2013/036358**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2003/057847 A2	17 Jul 2003	EP 1478655 A2	24 Nov 2004
		US 2008027015 A1	31 Jan 2008
		WO 03057847 A2	17 Jul 2003
WO 2010/062817 A1	03 Jun 2010	CA 2744694 A1	03 Jun 2010
		CN 102292443 A	21 Dec 2011
		EA 201100847 A1	30 Jan 2012
		EP 2370578 A1	05 Oct 2011
		JP 2012509688 A	26 Apr 2012
		KR 20110095391 A	24 Aug 2011
		MX 2011005576 A	17 Aug 2011
		US 2011288154 A1	24 Nov 2011
		WO 2010062817 A1	03 Jun 2010
WO 2011/044167 A1	14 Apr 2011	None	
WO 2007/071369 A2	28 Jun 2007	None	
WO 2009/074575 A2	18 Jun 2009	None	

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2013/036358**

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Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.