Abstract: In accordance with the present invention, it has been discovered that PBIF is an effective agent for inducing and/or augmenting the labor process, and related conditions (e.g., preparing the uterus for inducing labor, inducing cervical ripening, promoting pregnancy termination, treating incomplete miscarriage, treating post partum hemorrhage, and the like). Accordingly, methods are provided for preparing the uterus for inducing labor, inducing cervical ripening, promoting pregnancy termination, treating incomplete miscarriage, treating post partum hemorrhage, and the like. In addition, methods are also provided for treating ectopic or abdominal pregnancy. The previously observed action of PBIF, i.e., to cause the upregulation of other genes which code for proteins involved in starting the labor process, is very different from its direct action on the myometrium observed herein. What was previously demonstrated by microarray analysis (Ognjanovic S, Tashima LS, Bryant-Greenwood GD. The effects of pre-B-cell colony enhancing factor on the human fetal membranes by microarray analysis. Amer J Obstet Gynecol (2003) 189:1187-1195) was that in four hours, the PBIF acted upon the fetal membrane, which surrounds the inside of the uterus, to upregulate this important cassette of genes. The proteins encoded by these genes would then be made by the cells of the fetal membrane and their secretion would allow them to act upon the nearby tissues, the uterus and cervix. On the other hand, the discovery being addressed herein shows a direct and almost instant effect of PBIF on human myometrial contraction. This action is surprising in that it is rapid and direct, and therefore cannot be occurring via the upregulation of other genes and the action of their proteins.
METHODS FOR INDUCING UTERINE CONTRACTION AND COMPOSITIONS USEFUL THEREFOR

FIELD OF THE INVENTION

[0001] In one aspect, the present invention relates to methods for inducing or augmenting labor and/or cervical ripening. In another aspect, the present invention relates to methods for promoting pregnancy termination. In a further aspect, the present invention relates to methods for treating incomplete miscarriage and/or post partum hemorrhage. In yet another aspect, the present invention relates to methods for treating ectopic or abdominal pregnancy.

BACKGROUND OF THE INVENTION

[0002] In obstetrics it is often required to initiate the contraction of the uterus, either for the initiation or augmentation of labor in women or to induce abortion. The synthetic form of the octapeptide hormone, oxytocin, is commercially available in the United States under the trade names: Syntocinon and Pitocin and is used for this purpose. The use of this hormone suffers from several limitations and drawbacks, e.g., it is not effective by mouth and has to be administered intravenously. Its half-life in the blood is very short, approximately 3 minutes. Additional potential problems include, for example, the fact that before delivery, the spontaneously laboring uterus is extremely sensitive to oxytocin and with an inappropriate dose, the pregnant uterus may contract so violently that it can kill the fetus or rupture the uterus (Williams Obstetrics. 2001 215t Edition. McGraw Hill, New York). Deleterious effects can also follow the intravenous injection of oxytocin, for example, it has been shown even in healthy women to cause a transient but marked fall in arterial blood pressure that was followed by an abrupt increase in cardiac output. Therefore warnings have been issued that oxytocin should not be given as an intravenous bolus in large amounts, but as a dilute slow infusion or an intramuscular injection. Oxytocin can also be used to induce a mid-trimester abortion utilizing its action to cause uterine contraction, the same precautions
apply to this use. Accordingly, the identification of additional agents which can be effectively used for the initiation or augmentation of labor in women or to induce abortion would be of great interest.

[0003] Pre-B-cell colony enhancing factor (PBEF) was first identified from activated peripheral blood lymphocytes and shown to be involved in the maturation of B-cell precursors (see Samal B, Sun Y, Stearns G, Xie C, Suggs S and McNiece I. “Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor.” in Mol Cell Biol 1994;14:1431-7). In studying the effects of acute distension on the genes expressed by human amniotic epithelial cells (WISH), one of these genes was identified as PBEF (see Nemeth E, Tashima LS, Yu Z and Bryant-Greenwood GD. “Fetal membrane distension I. Differentially expressed genes regulated by acute distension in amniotic epithelial (WISH) cells.” in Am J Obstet Gynecol 2000;192:50-9; referred to hereinafter as “Nemeth I”). When pre-term and term full-thickness fetal membranes were similarly distended in vitro, it was shown that the expression of the PBEF gene was greater in the pre-term than in the term membranes (see Nemeth E, Millar LK and Bryant-Greenwood G. “Fetal membrane distension: II. Differentially expressed genes regulated by acute distension in vitro.” in Am J Obstet Gynecol 2000;182:60-7; referred to hereinafter as “Nemeth II”). This suggested that by term, the tissue had attained its maximum distension, and sensitivity to further distension was then limited. While distension is a part of the normal process of pregnancy, uterine contractions also cause distension, thus distension is also a key part of the labor process.

[0004] A genomic clone of PBEF was subsequently analysed and shown to be a highly regulated gene. An important finding in this study was the identification of an NF-kB binding element in the third intron, likely to be responsible for the responsiveness to distension (see Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, and Bryant-Greenwood GD. “Genomic organization of the gene coding for human pre-B cell colony-enhancing factor and expression in human fetal membranes.” in J Mol Endocrinol 2001;26:107-117). PBEF was also localized with a specific antiserum, showing that the
expression of the PBEF gene was significantly upregulated in severely infected membranes. In addition, the neutrophils present in these tissues stained darkly for PBEF, suggesting that they are an additional source of PBEF in the setting of infection (see Ognjanovic et al., supra). However, while LPS, TNF-α, IL-1β and IL-6 all induced PBEF gene expression, it has been shown that IL-8 treatment had no such effect (see Ognjanovic et al., supra). This was particularly interesting because IL-8 and PBEF expression both increased when WISH cells and the fetal membranes were acutely distended (see Nemeth I and Nemeth II, supra). Therefore, like PBEF, IL-8 appears to be responsive to distension in a sterile situation, as well as being induced by infection, but its relationship with PBEF under these conditions is unknown.

[0005] PBEF is present in fetal membranes during normal gestation and parturition in the absence of infection. Its expression increases with both pre-term labor and normal labor at term. Moreover, PBEF causes an increase in the expression in fetal membranes of many key proteins that are known activators of the labor process (see, for example, FIG. 1). Thus, it has previously been shown that PBEF expression is upregulated in the fetal membranes by both term and preterm labor and delivery (Ognjanovic S, Bryant-Greenwood GD. "Pre-B-cell colony enhancing factor, a novel cytokine of human fetal membranes" in Amer J Obstet Gynecol (2002) 187:1051-1058). Thus, the protein was added to squares of fetal membrane and DNA microarrays (Affymetrix) were used to show its action on this tissue (Ognjanovic S, Tashima LS, Bryant-Greenwood GD. "The effects of pre-B-cell colony enhancing factor on the human fetal membranes by microarray analysis" in Amer J Obstet Gynecol (2003) 189:1187-1195). This work showed that PBEF causes the upregulation of key genes from the fetal membranes involved in the initiation of labor. It has thus previously been determined that PBEF is a "driver" at the apex of the cytokine cascade, known to cause the start of labor in women.

[0006] It has now been investigated whether PBEF itself could serve as a useful agent for inducing and/or augmenting the labor process, and related conditions (e.g., cervical ripening, incomplete miscarriage, post partum hemorrhage, and the like), thereby providing
new treatment modalities not previously available. Furthermore, it was investigated whether the action of PBEF could be blocked, or its expression blocked, thereby stopping the labor process and providing new treatment modalities not previously available.

SUMMARY OF THE INVENTION

[0007] In accordance with the present invention, it has been discovered that PBEF is an effective agent for inducing and/or augmenting the labor process, and related conditions (e.g., preparing the uterus for inducing labor, inducing cervical ripening, promoting pregnancy termination, treating incomplete miscarriage, treating post partum hemorrhage, and the like). Accordingly, methods are provided for preparing the uterus for inducing labor, inducing cervical ripening, promoting pregnancy termination, treating incomplete miscarriage, treating post partum hemorrhage, and the like. In addition, methods are also provided for treating ectopic or abdominal pregnancy. The use of PBEF to cause uterine contraction in women has the potential to avoid some of the side effects of oxytocin. This is based upon the known and comparative physiology of these two proteins. The synthesis of oxytocin does not occur within the myometrial muscle itself, while PBEF appears to be expressed by this tissue. In addition, it has been shown that PBEF gene expression is significantly increased in the fetal membranes of women in both term and preterm labor, while the expression of oxytocin in the normal initiation of human parturition, does not manifest at least until the second stage of labor.

[0008] The previously observed action of PBEF, i.e., to cause the upregulation of other genes which code for proteins involved in starting the labor process, is very different from its direct action on the myometrium observed herein. What was previously demonstrated by microarray analysis (Ognjanovic S, Tashima LS, Bryant-Greenwood GD. “The effects of pre-B-cell colony enhancing factor on the human fetal membranes by microarray analysis” in Amer J Obstet Gynecol (2003) 189:1187-1195) was that in four hours, the PBEF acted upon the fetal membrane, which surrounds the inside of the uterus, to upregulate this important cassette of genes. The proteins encoded by these genes would then
be made by the cells of the fetal membrane and their secretion would allow them to act upon the nearby tissues, the uterus and cervix.

[0009] On the other hand, the discovery being addressed herein shows a direct and almost instant effect of PBEF on human myometrial contraction. This action is surprising in that it is rapid and direct, and therefore cannot be occurring via the upregulation of other genes and the action of their proteins.

**BRIEF DESCRIPTION OF THE FIGURES**

[0010] **FIG. 1** demonstrates the effect of rhPBEF treatment on the expression of IL-8 in fetal membrane explants. RhPBEF (100 ng/ml) caused *significant* (p<0.05) increase in IL-8 gene expression after 4h of treatment, compared to the control.

[0011] **FIG. 2** presents a diagrammatic summary of the possible relationships between PBEF, IL-6 and IL-8, and their controls by mechanical stimulation, by infectious stimulation and by labor.

[0012] **FIG. 3** is illustrative of the effect of PBEF on the contraction activity of human myometrium obtained from late pregnant subjects not yet in labor.

**DETAILED DESCRIPTION OF THE INVENTION**

[0013] In accordance with the present invention, there are provided methods for preparing the uterus for inducing labor or for pregnancy termination in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to induce labor. Invention methods are useful in a variety of circumstances, especially when the subject being treated has reached term and has not yet spontaneously commenced labor or when the subject being treated has commenced
labor but would benefit from assistance to complete the labor process.

[0014] As readily recognized by those of skill in the art, subjects in need of the above-described treatments can be selected from subjects in need of elective induction of labor, subjects experiencing incomplete miscarriage, subjects experiencing post-partum hemorrhage, subjects in need of labor augmentation, and the like.

[0015] Depending on the mode of delivery employed, the compounds contemplated for use herein can be delivered in a variety of pharmaceutically acceptable forms. For example, compounds contemplated for use in the practice of the present invention can be delivered in the form of a solid, solution, emulsion, dispersion, micelle, liposome, and the like.

[0016] Thus, in accordance with still another embodiment of the present invention, there are provided pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

[0017] Pharmaceutical compositions of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting composition contains one or more of the compounds contemplated for use in the practice of the present invention as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for enteral or parenteral applications. Invention compounds may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions, and any other form suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides,
dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. Invention compounds are included in the pharmaceutical composition in an amount sufficient to produce the desired effect upon the process or disease condition.

[0018] Pharmaceutical compositions containing compounds contemplated for use in the practice of the present invention may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing compounds contemplated for use in the practice of the present invention, in admixture with non-toxic pharmaceutically acceptable excipients, may also be manufactured by known methods. The excipients used may be, for example, (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such as corn starch, potato starch or alginic acid; (3) binding agents such as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

[0019] In some cases, formulations for oral use may be in the form of hard gelatin capsules wherein compounds contemplated for use in the practice of the present invention are
mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein compounds contemplated for use in the practice of the present invention are mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

[0020] The pharmaceutical compositions may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids (including oleic acid), naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc., or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

[0021] Compounds contemplated for use in the practice of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions may be prepared by mixing the invention compounds with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

[0022] Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise mode of administration and dosage employed for each subject is left to the discretion of the practitioner. In general, the dosage of invention compounds employed as described herein falls in the range of about 0.01 mmoles/kg body weight of the subject/hour up to about 0.5 mmoles/kg/hr. Typical daily doses, in general, lie within the range of from about 10 μg up to about 100 mg per kg body weight, and, preferably within the range of from 50 μg to 10 mg.
per kg body weight and can be administered up to four times daily. The daily IV dose lies within the range of from about 1 µg to about 100 mg per kg body weight, and, preferably, within the range of from 10 µg to 10 mg per kg body weight.

[0023] In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 µg/ml.

[0024] In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

[0025] In accordance with another embodiment of the present invention, there are provided methods of inducing cervical ripening in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to induce cervical ripening.

[0026] As readily recognized by those of skill in the art, subjects in need of the above-described treatments can be selected from subjects in need of elective induction of labor, subjects experiencing incomplete miscarriage, subjects experiencing post-partum hemorrhage, subjects in need of labor augmentation, subjects who have commenced pre-term labor or spontaneous abortion, and the like.

[0027] In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 µg/ml.
Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

In accordance with still another embodiment of the present invention, there are provided methods for promoting pregnancy termination in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote pregnancy termination.

As readily recognized by those of skill in the art, subjects in need of the above-described treatments can be selected from subjects in need of elective induction of labor, subjects experiencing incomplete miscarriage, subjects experiencing post-partum hemorrhage, subjects in need of labor augmentation, and the like.

In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery,
topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

[0034] In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

[0035] In accordance with a further embodiment of the present invention, there are provided methods for treating incomplete miscarriage in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote delivery of the placenta.

[0036] As readily recognized by those of skill in the art, subjects in need of the above-described treatments can be selected from subjects experiencing incomplete miscarriage, pregnancy termination for medical conditions, and the like.

[0037] In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

[0038] Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.
In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

In accordance with a still further embodiment of the present invention, there are provided methods for treating post partum hemorrhage in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to cause the uterus to contract, thereby closing off blood vessels and reducing blood loss.

In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

In accordance with yet another embodiment of the present invention, there are provided methods for augmenting labor in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF)
effective to augment labor.

[0045] As readily recognized by those of skill in the art, subjects in need of the above-described treatments can be selected from subjects who fail to advance through the normal steps of labor, subjects in post-term pregnancy, pregnancy termination for medical conditions, and the like.

[0046] In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

[0047] Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

[0048] In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

[0049] In accordance with still another embodiment of the present invention, there are provided methods for treating ectopic or abdominal pregnancy in a subject in need thereof, said methods comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote uterine contraction.

[0050] In a particular embodiment of the present invention, the effective amount of PBEF contemplated for use herein is determined by the uterine response of the specific
individual being treated. Typically for this embodiment of the present invention, the effective amount of PBEF falls in the range of about 0.1-10 µg/ml.

[0051] Thus, in accordance with this embodiment of the present invention, pharmaceutical formulations comprising compounds suitable for use in the practice of the present invention are typically delivered in a suitable vehicle rendering said compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, intraamniotically, intrauterine delivery, intraperitoneally, and the like.

[0052] In addition to use of PBEF, combinations of agents, such as PBEF plus oxytocin, pitocin, prostaglandins, and the like, as well as combinations of any two or more thereof, can also be employed in the practice of the present invention.

[0053] Recombinant human PBEF (rhPBEF) was produced in order to study the relationship of PBEF with other key cytokines and proteins, which are known to be inducers of uterine contractions and/or cervical dilatation. In one study which involved adding PBEF to either WISH cells or explants of fetal membrane, it was shown that PBEF increased the expression of mRNA for IL-6 in a dose-dependent manner (see Ognjanovic et al., supra). In explants, PBEF increased the expression of the IL-6 gene by 40%, which approximated the maximal effect of PBEF (44%) in WISH cells. In a similar way it was shown that PBEF caused a 120% increase in the expression of IL-8 in fetal membrane explants.

[0054] The proinflammatory cytokines TNF-α and IL-1β have been shown to stimulate IL-6 (see Keelan JA, Sato T, and Mitchell MD. “Regulation of interleukin-6 and interleukin-8 production in an amnion-derived cell line by cytokines, growth factors, glucocorticoids and phorbol esters.” in Am J Reprod Imm 1997;38:272-8), IL-8 (see, for example, Keelan et al., supra; and Laham N Brennecke SP and Rice GE. “Interleukin-8 release from human gestational tissue explants: the effects of lipopolysaccharide and cytokines.” in Biol. Reprod. 1997; 57:616-20) and PBEF (see Ognjanovic et al., supra)
transcription. It has also been demonstrated that rhPBEF stimulates the expression of TNF-α, IL-1β, IL-6, and cyclooxygenase-2 (COX-2) gene expression in the fetal membranes. This clearly places PBEF at the upstream point of the known pathway to labor, at pre-term or term (see FIG. 2). These studies were conducted with rhPBEF addition to explants of fetal membranes and the RNA from treated and control (untreated) tissue used for isolation of total RNA and hybridization to DNA microchips (Affymetrix Inc.). Computer analysis of the signals identified 7 genes in the labor cascade which were upregulated in the fetal membranes by their treatment with rhPBEF, in all patients studied. This has been confirmed in both tissue from other patients and by measurement of the respective secreted proteins in addition to the expression of the genes in the tissues. Thus, PBEF is an initiator of the cascade of events currently recognized as leading to labor and delivery.

[0055] The invention will now be described in greater detail by reference to the following non-limiting examples.

**EXAMPLE 1**

Recombinant human PBEF protein production

[0056] rhPBEF was produced in a bacterial system using pTrcHis2 vector (Invitrogen, Carlsbad, CA). PBEF was amplified by polymerase chain reaction (PCR) from a library prepared from fresh human amnion, chorion and decidua with 5’ and 3’ primers:

CAACAAAGAATTCATGAATCTGCGGAGAAG (SEQ ID NO:1), and
CTTAAGCGCAGGCGATGTGTGCTTGTTCCAGTTC (SEQ ID NO:2), respectively. The PCR product was subcloned in the pTrcHis2 vector and positive colonies were identified by colony lift. One clone was sent for sequencing at the University of Hawaii Biotechnology Facility and the rhPBEF sequence was confirmed. The production of the rhPBEF protein was carried out following the Invitrogen pcDNA 3.1/V5-His TOPO TA Cloning Kit protocol. Briefly, a single colony was used to inoculate Luria-Bertani (LB)
media and the culture was incubated until the optical density at OD<sub>600</sub> reached 0.6, when rhPBEF production was induced with 50 mM isopropyl β-D-thiogalactopyranoside (IPTG). It was stopped after 8h, the cells were then collected by centrifugation and lysed by freeze-thawing (alternating methanol-dry-ice and 37°C waterbath). The lysates were spun, filtered through 0.8 μm syringe filters and stored at −20°C. The rhPBEF protein was purified using ProBond resin from Xpress System Protein Purification (Invitrogen,) according to the manufacturer’s manual. Briefly, the resin was equilibrated with native binding buffer (20mM phosphate buffer 500mM NaCl, pH 7.8) and the lysates loaded. The washes were carried out in native wash buffer (20mM phosphate buffer 500mM NaCl, pH 6.0) and rhPBEF was eluted with 350 mM imidazole. All eluates were collected, imidazole was replaced with 50 mM sodium phosphate buffer pH 7.2 and concentrated using Centricon Plus-20 filtration units (Millipore Corporation, Bedford, MA). The concentration of rhPBEF was determined using the BioRad Protein Assay (BioRad Laboratories, Hercules, CA). An aliquot (50 μg) was kindly sequenced by Dr E. Petricoin, FDA/NIH Washington DC, and six separate peptides confirmed its identity. In addition, an aliquot was assayed for lipopolysaccharide content by Biowhittaker Inc. (Walkersville, MD). This showed a concentration of 0.03 EU/ng, well below the acceptable limit of 10EU/ng.

**EXAMPLE 2**

Cell contraction studies

[0057] Small pieces of human uterine muscle (myometrium) were obtained (with informed written consent) from pregnant women at the time of elective Caesarian section before labor had commenced. The muscle strips are suspended in a warmed tissue bath, where the tissue spontaneously continues to regularly contract, and the resulting contractions recorded. The PBEF (or control substance, or comparative substance) is then added to the pieces of muscle in small doses.

[0058] PBEF is observed to have a very marked effect on the human muscle, causing it to contract very rapidly, even after it had been given other standard agents which cause
contraction (see, for example, FIG. 3). In contrast to this action on human myometrium, PBEF was not observed to have any effect on the contraction of rat myometrium. This represents a dramatic illustration of that which is known in the art, i.e., that the control of myometrial contraction in the rat and human is different.

**EXAMPLE 3**

PBEF Assays

[0059] PBEF levels can be measured in biological samples in a variety of ways. For example, an ELISA can be carried out using antibodies raised against recombinant human PBEF or against synthetic peptides derived from PBEF and raised in sheep, against recombinant human PBEF or synthetic peptides based on PBEF and raised in rabbits, against recombinant human PBEF or against synthetic peptides based on PBEF and raised in chickens, and the like.

[0060] In one aspect, the ELISA can be carried out in a competitive format by coating ELISA plates with anti-PBEF antibodies, blocking the non-specific sites with blocking buffer and incubating them with different amounts of biotinylated recombinant human PBEF protein (biotin-rhPBEF) to provide the standard curve. Experimental samples or unknowns are then diluted sequentially and added to the known amount of biotin-rhPBEF. The standards and unknowns are then incubated with streptavidin-conjugated horseradish peroxidase (HRPO) and the amount of biotin-rhPBEF detected by color development with a color forming compound such as 2,2-azino-bis(3-ethylbenzthiazoline-6 sulfonic acid). Displacement of the biotin-rhPBEF by the PBEF protein is measured by the amount of color developed—the color is directly related to the amount of PBEF present in the sample. The amount of PBEF in the unknown samples can then be quantitated against the standard curve.

[0061] If desired, the sensitivity of the above-described assay can be enhanced by such techniques as signal amplification, use of alkaline phosphatase (in place of HRPO), along with a fluorescent substrate such as 4-methylumbelliferyl phosphate, and the like.
While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.
That which is claimed is:

1. A method for preparing the uterus for inducing labor or for pregnancy termination in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to induce labor.

2. The method of claim 1 wherein said subject in need thereof is selected from the group consisting of a subject in need of an elective induction of labor, a subject experiencing incomplete miscarriage, a subject experiencing post-partum hemorrhage and a subject in need of labor augmentation.

3. The method of claim 1 wherein an effective amount of PBEF is determined by the uterine response.

4. The method of claim 3 wherein an effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

5. The method of claim 1 wherein said effective amount of PBEF is administered by oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, subcutaneous injection, intracervically, sublingually, nasally, intraamniotic, intrauterine delivery, or intraperitoneally.

6. A method of inducing cervical ripening in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to induce cervical ripening.

7. The method of claim 6 wherein said subject in need thereof is selected from the group consisting of a subject in need of an elective induction of labor, a subject experiencing incomplete miscarriage, a subject experiencing post-partum hemorrhage and a subject in need of labor augmentation.
8. The method of claim 6 wherein an effective amount of PBEF is determined by the uterine response.

9. The method of claim 8 wherein an effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

10. The method of claim 6 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamniotically, intrauterine, or intraperitoneally.

11. The method of claim 6 wherein said PBEF is administered in combination with one or more agents selected from the group consisting of oxytocin, pitocin, and prostaglandins.

12. The method of claim 6 wherein said subject has commenced pre-term labor or spontaneous abortion.

13. A method for promoting pregnancy termination in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote pregnancy termination.

14. The method of claim 13 wherein said subject in need thereof is selected from the group consisting of a subject in need of an elective induction of labor, a subject experiencing incomplete miscarriage, a subject experiencing post-partum hemorrhage and a subject in need of labor augmentation.

15. The method of claim 11 wherein an effective amount of PBEF is determined by the uterine response.
16. The method of claim 15 wherein an effective amount of PBEF falls in the range of about 0.1-10 µg/ml.

17. The method of claim 11 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamnionically, intrauterine, or intraperitoneally.

18. A method for treating incomplete miscarriage in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote delivery of the placenta.

19. The method of claim 18 wherein said subject in need thereof is a subject experiencing incomplete miscarriage.

20. The method of claim 18 wherein an effective amount of PBEF is determined by the uterine response.

21. The method of claim 20 wherein an effective amount of PBEF falls in the range of about 0.1-10 µg/ml.

22. The method of claim 18 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamnionically, intrauterine, or intraperitoneally.

23. A method for treating post partum hemorrhage in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to cause the uterus to contract, thereby closing off blood vessels and reducing blood loss.
24. The method of claim 23 wherein an effective amount of PBEF is determined by the uterine response.

25. The method of claim 24 wherein an effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

26. The method of claim 23 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamniotically, intrauterine, or intraperitoneally.

27. A method for augmenting labor in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to augment labor.

28. The method of claim 27 wherein said subject in need thereof is a subject who fails to advance through the normal steps of labor.

29. The method of claim 27 wherein an effective amount of PBEF is determined by the uterine response.

30. The method of claim 29 wherein an effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

31. The method of claim 27 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamniotically, intrauterine, or intraperitoneally.
32. A method for treating ectopic or abdominal pregnancy in a subject in need thereof, said method comprising administering to said subject an amount of pre-B cell colony-enhancing factor (PBEF) effective to promote uterine contraction.

33. The method of claim 32 wherein an effective amount of PBEF falls in the range of about 0.1-10 μg/ml.

34. The method of claim 32 wherein said effective amount of PBEF is administered intravenously, by intramuscular injection, by subcutaneous injection, by oral administration, intracervically, sublingually, nasally, intraamniotic, intrauterine, or intraperitoneally.
FIG. 1
FIG. 2
Human myometrium – late pregnant, nonlabor

FIG. 3

KCl
Wash
PBEF 1
μg/ml
PBS buffer
Wash