Title: TGF-BETA COMPOSITIONS FOR TREATING INFERTILITY

Abstract: This invention relates to pharmaceutical compositions of members of the TGF superfamily, and especially to compositions comprising members of the mammalian TGFβ superfamily, in which the composition comprises a substantially purified TGFβ, a zwitterion, and a pharmaceutically acceptable carrier. The zwitterion may be selected from the group consisting of an amino acid, a zwitterionic buffer compound, a zwitterionic detergent compound and an alkaloid. In some embodiments the invention relates to a composition comprising TGFβ, which is stable at pH 2.0 to 7.0 for at least 6 months at a temperature between 4°C and 27°C.
TGF-beta compositions for treating infertility

FIELD

This invention relates to pharmaceutical compositions of members of the TGF superfamily, and especially to compositions comprising members of the mammalian TGFβ superfamily.

INTRODUCTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Transforming growth factors (TGFs) are a group of multifunctional cytokines which play an important role in the development and repair of tissue. TGFβ belongs to a large family of related factors, referred to as the TGFβ superfamily, which share at least 25% sequence homology. Members of the TGFβ superfamily affect multiple physiological processes, including cell cycle progression, cell survival and differentiation during prenatal development and in the adult organism, and modulation of immune responses.

The superfamily of TGFβs includes over 30 members, which are divided into several groups, including the TGFβ-related dimeric proteins (e.g. TGFβ1 - TGFβ5), Mullerian inhibitory substances (e.g. MIS), bone morphogenetic proteins (e.g. BMP-2-7), inhibins and activins (e.g. inhibin A, inhibin B, activin A and activin AB), growth differentiation factors (e.g. GDF-1), dorsalin-1 (e.g. dsl-1), MIC-1 and Drosophila decapentaplegic gene product (e.g. DPP-C).

All members of the TGFβ superfamily show amino acid sequence similarity to the prototype TGFβ1 isoform, with a conserved tertiary structure which includes nine highly conserved cystine residues forming a cystine knot via disulphide-linked dimers.

The mammalian TGFβ subfamily consists of five isoforms with similar structure and function, i.e. TGFβ1, TGFβ2, TGFβ3, TGFβ4 and TGFβ5. TGFβ1, TGFβ2 and TGFβ3 isoforms are expressed in mammalian cells, while TGFβ4
and TGFβ5 represent a chicken and a *Xenopus laevis* TGFβ isoform, respectively. They form homodimeric proteins with a molecular weight of 25,000 Daltons. The mature peptide sequences of the five isoforms of TGFβs isolated to date have 64-82% similarity at the amino acid level, including the conserved cystine knot structure.

The TGFβ subfamily isoforms possess similar biological activities, and act through similar signaling cascades, which are initiated by binding of TGFβ to heteromeric complexes of type I and type II TGFβ receptors at the surface of target cells (Wrana et al., 1994). To elicit biological effects, the TGFβ isoforms which are in a latent form must become activated after initial secretion (Khalil, 1999).

It is desirable that any pharmaceutical compositions which contain TGFβ as the active agent are stable for long periods of time, that the TGFβ retains bioactivity and is bioavailable, and that the composition is ready to administer in liquid form, and does not require reconstitution of lyophilized TGFβ immediately before use. However, researchers have encountered many problems in developing pharmaceutical compositions containing TGFβ. These include:

1. TGFβ in solution can aggregate. If aggregation is present, the TGFβ may not be biologically active, and may result in undesired immunological effects.

2. TGFβ has a high affinity for surfaces of containers and equipment, especially those made of glass or plastic, and therefore TGFβ is lost by adsorption when in solution, particularly at low concentrations. This is a major problem, because it results in reduced yields during manufacture of the TGFβ active agent, as the protein will adhere to equipment and lines, thus increasing the complexity and cost of manufacture. Moreover, measuring the concentration of the protein in the drug product is difficult, because the protein will adhere to vials and instruments, thus resulting in an inaccurate determination of drug concentration. This undesired property of adherence of TGFβ to surfaces also reduces the actual dose delivered to the patient.

3. TGFβ is unstable when in solution under conditions currently used in the art, because it readily undergoes deamidation and oxidation. Consequently bioactivity is reduced, and degradation products lead to increased aggregation, further reducing bioactivity.

4. TGFβ has a low aqueous solubility under non-optimised conditions. If TGFβ precipitates, bioactivity can be lost or become unpredictable.
(5) The maximum pH at which TGFβ is soluble is 3.8 (Pellaud J et al., J. Biol. Chem. (1999) 274; 7699-7704). Above this pH, TGFβ precipitation is caused by the aggregation of TGFβ in solution due to the molecule's normal charge, which leads to association of the TGFβ dimers, ultimately leading to aggregation and precipitation. TGFβ formulations at this pH are prone to cause irritation at the delivery site, especially on topical administration.

(6) If the pharmaceutical composition comprising the TGFβ is intended for delivery to a mucosal surface, for example intra-vaginal delivery to the cervix or vagina, the composition should not irritate the mucosal surface.

(7) If the pharmaceutical composition comprising the TGFβ is intended for intra-vaginal delivery to the cervix for the treatment of an infertility condition, the composition should not be toxic to sperm, or affect conception rates.

International Patent Application No. PCT/EPOO/02303 describes a dry powder pharmaceutical composition comprising TGFβ and a water-soluble salt chosen from calcium chloride and calcium phosphate. PCT/EPOO/02303 proposes that the problems associated with the poor physical and chemical stability of TGFβ and its high adherence to surfaces of containers can be reduced by the addition of one of these specifically-listed water-soluble salts. However, the present inventors have found that this composition does not in fact provide a stable solution; indeed, they have found that singly- or doubly-charged ions actually induce precipitation.

Therefore there remains a need in the art for TGFβ formulations:

(1) which are stable; and/or
(2) wherein the TGFβ protein remains soluble above a pH of 3.8; and/or
(3) wherein the TGFβ does not adhere to the surfaces of containers; and/or
(4) which do not irritate mucosal surfaces such as the vagina; and/or
(5) which are not toxic to sperm.

SUMMARY OF THE INVENTION

The inventors have now developed a composition comprising TGFβ which is stable at pH 2.0 to 7.0 for at least 6 months at a temperature between 4° and 27°C.

The inventors have found that when a liquid TGFβ solution is formulated with a zwitterion, the TGFβ remains soluble even when in solution at neutral pH. Zwitterions reduce TGFβ aggregation, which is the cause of protein precipitation, and does so at pHs above 3.7. Without wishing to be bound by
theory, the zwitterions are thought to achieve this by protecting the TGFβ from forming an aggregated state by intermolecular association, it is thought that this physicochemical property of zwitterions neutralizes the unbalanced charge of TGFβ, preventing association with other TGFβ molecules, and thus preventing aggregation and precipitation. Formulation of TGFβ in liquid form at pH above 3.7 has many advantages, as discussed below.

The inventors have found that in contrast to zwitterions, a formulation with a monovalent or divalent counter-cation, such as sodium or calcium, and/or an anion, such as acetate, chloride or citrate, does not confer solubility. In fact these charged ions have the reverse effect, and actually destabilise TGFβ, leading to higher aggregation and precipitation.

In a first aspect the invention provides a composition comprising a substantially purified TGFβ, a zwitterion, and a pharmaceutically acceptable carrier.

In one embodiment the zwitterion is selected from the group consisting of an amino acid, a zwitterionic buffer compound, a zwitterionic detergent compound and an alkaloid.

The amino acid may be any of the common or uncommon amino acids, and for example is selected from the group consisting of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine and tyrosine, and analogues thereof, e.g. norleucine, noralanine, norvaline, homoarginine, homolysine etc. In one embodiment the amino acid is selected from the group consisting of arginine and glycine. Most preferably the amino acid is glycine.

Zwitterionic buffers, also known as Good's buffers (Good et al., 1966, Biochemistry 5: 467; Good and Izawa 1972, Methods in Enzymol. 24: 62), are well known in the art, and are commercially available from many sources; a zwitterionic buffer can be readily chosen for a given pH. For example, the zwitterionic buffer compound may be selected from the group consisting of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pipazine-N,N'-bis(2-ethanesulfonic acid)(PIPS), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), and 3-(N-morpholino)propanesulfonic acid (MOPS).

Preferably the zwitterionic detergent compound is selected from the group consisting of 3-[[3-Cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), 3-[[3-Cholamidopropyl]dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), 3-
(Decyldimethylammonio)propanesulfonate inner salt, 3-(Dodecyldimethylammonio)propanesulfonate inner salt, 3-(N,N-Dimethylmyristylammonio)propanesulfonate, 3-(N,N-Dimethyloctadecylammonio)propanesulfonate, 3-(N,N-Dimethyloctylammonio)propanesulfonate inner salt, and 3-(N,N-Dimethylpalmitylammonio)propanesulfonate.

Preferably the alkaloid is a pharmacologically inert compound selected from the group consisting of a pyridine, a pyrrolidine, a tropane, a quinoline, an isoquinoline, a phenethylamine, an indole, an ergoline, a beta-carboline, a purine group or derivative thereof, a terpenoid, a steroid, a betaine, and a pyrazole.

The physiologically acceptable carrier may be an aqueous pH buffered solution, and may include buffering agents such as phosphate, citrate, and other organic acids; preservative agents such as benzoic acid; antioxidants such as ascorbic acid; less than about 10 amino acid residue peptides of proteins, such as serum albumin or gelatin; gel polymers; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or dextrins; chelating agents such as EDTA; osmotic agents such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants, such as Tween, polyethylene glycol (PEG), and Pluronics™.

In one embodiment the pH of the composition is between 2.0 and 7.0. Preferably the pH of the composition is 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, or 7.0.

In a further embodiment the pH of the composition is between 3.7 and 7. Preferably the pH of the composition is between 3.7 and 5.5. For example, the pH of the composition may be between 3.7 and 5.2, 3.7 and 5.0, or 3.7 and 4.8. Still further, the pH of the composition may be between 3.8 and 7, 3.8 and 5.5, 3.8 and 5.2, 3.8 and 5.0, or 3.8 and 4.8. Still further, the pH of the composition may be between 4.0 and 7, 4.0 and 5.5, 4.0 and 5.2, 4.0 and 5.0, or 4.0 and 4.8. Most preferably the pH of the composition is 4.8, 5.0, 5.2, or 5.5.

In one embodiment, the amino acid, zwitterionic buffer compound, zwitterionic detergent compound, or alkaloid is at a concentration of between 0.01% and 1.0% w/w. Preferably the concentration of the amino acid, zwitterionic buffer compound, zwitterionic detergent compound, or alkaloid is
0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, 0.20%, 0.21%, 0.22%, 0.23%, 0.24%, 0.25%, 0.26%, 0.27%, 0.28%, 0.29%, 0.30%, 0.31%, 0.32%, 0.33%, 0.34%, 0.35%, 0.36%, 0.37%, 0.38%, 0.39%, 0.40%, 0.41%, 0.42%, 0.43%, 0.44%, 0.45%, 0.46%, 0.47%, 0.48%, 0.49%, 0.50%, 0.51%, 0.52%, 0.53%, 0.54%, 0.55%, 0.56%, 0.57%, 0.58%, 0.59%, 0.60%, 0.61%, 0.62%, 0.63%, 0.64%, 0.65%, 0.66%, 0.67%, 0.68%, 0.269%, 0.70%, 0.71%, 0.72%, 0.73%, 0.74%, 0.75%, 0.76%, 0.77%, 0.78%, 0.279%, 0.80%, 0.81%, 0.82%, 0.83%, 0.84%, 0.85%, 0.86%, 0.87%, 0.88%, 0.89%, 0.90%, 0.91%, 0.92%, 0.93%, 0.94%, 0.95%, 0.96%, 0.97%, 0.98%, 0.99%, or 1.0% w/w. Most preferably the amino acid, zwitterionic buffer compound, zwitterionic detergent compound or alkaloid is at a concentration of 0.15% w/w.

In one embodiment the composition is in a liquid form. In a further embodiment the composition does not require reconstitution immediately prior to use. In a still further embodiment the composition does not undergo a step of freeze-drying. Preferably, the TGFβ component does not undergo a step of freeze-drying. Preferably the composition is in a form ready to administer. The composition may be in a form suitable for administration by a patient.

In a second embodiment the composition comprises a pharmaceutically acceptable gel polymer. Preferably the zwitterion is formulated in the gel polymer.

Preferably the gel polymer is selected from the group consisting of cellulose-based polymers (dispersible, microcrystalline and derivatives and semi-synthetic), tragacanth polymers (plant-derived), xanthan gum polymers (plant-derived), acacia polymers (plant-derived), carboxom polymers (carbopol) (acrylic acid polymer, synthetic), gelatin polymers (derived from animal collagen), sodium alginate polymers (algae extract), poloxomer polymers, polyethylene oxide polymers, polacrylamide polymers, polyethylene glycol polymers (macrogels), and carmellose sodium (high viscosity grade).

Preferably the gel polymer is a cellulose-based polymer. Preferably the cellulose-based polymer is selected from the group consisting of hypromellose (hydroxypropylmethylcellulose), methylcellulose, hydroxypropylcellulose, ethylcellulose, hydroxyethylcellulose and carboxymethylcellulose.

Preferably the cellulose-based polymer is capable of hydrogen bonding, and thus is not methylcellulose or ethylcellulose. More preferably the cellulose-based polymer contains a hydroxyl group, which includes hydroxypropylcellulose, hydroxyethylcellulose and hypromellose. Alternatively
the cellulose-based polymer contains a carboxy group, which includes carboxymethylcellulose. Preferably the gel polymer is methylcellulose with a grade of 4000, hydroxyethylcellulose with a grade of 4400, or hydroxypropyl cellulose with a grade of 4000. More preferably the gel is hypromellose and has a grade of 4000.

Preferably the gel polymer is at a concentration of between 1.0% and 5.0% w/w. More preferably the gel polymer is at a concentration of 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2.0%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3.0%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, 4.0%, 4.1%, 4.2%, 4.3%, 4.4%, 4.5%, 4.6%, 4.7%, 4.8%, 4.9% or 5.0% w/w. Even more preferably the gel polymer is at a concentration of 1.9% w/w. If a cellulose-based gel polymer is used, the polymer preferably contains a hydroxy group and/or a carboxy group.

In one embodiment the viscosity of the composition is between 200 and 30,000 cP and preferably between 500 and 900 cP. For example the viscosity of the composition is 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, or 700 cP.

In a further embodiment the composition does not contain calcium chloride or calcium phosphate. Preferably the composition does not contain calcium chloride, calcium phosphate, potassium acetate, lithium acetate, ammonium acetate or ammonium bicarbonate. Even more preferably the composition does not contain calcium chloride, calcium phosphate, potassium acetate, lithium acetate, sodium acetate, ammonium acetate or ammonium bicarbonate. Preferably calcium chloride, calcium phosphate, sodium acetate, potassium acetate, lithium acetate, ammonium acetate or ammonium bicarbonate is not added to the composition at any time during its manufacture. More preferably calcium chloride, calcium phosphate, potassium acetate, lithium acetate, ammonium acetate or ammonium bicarbonate is not added to the composition at any time during its manufacture. Most preferably calcium chloride or calcium phosphate is not added to the composition at any time during its manufacture.

In one embodiment, the TGFβ maintains stability and/or solubility and/or bioactivity for at least 6 months when stored at between 2° and 27°C. For example, the TGFβ maintains high bioactivity, low aggregation, low precipitation, low surface adherence and/or low oxidation and/or low deamidation for at least 6 months under these conditions. Alternatively the TGFβ maintains stability and/or solubility and/or bioactivity for at least 6 months.
when stored at between 2° and 8°C. Preferably the TGFβ maintains stability and/or solubility and/or bioactivity for at least 12 months when stored at between 2° and 27°C or between 2° and 8°C.

In another embodiment, the composition comprises a therapeutically effective amount of TGFβ. Preferably the concentration of TGFβ is between 0.01 µg/ml and 100 µg/ml. More preferably the concentration of TGFβ is between 0.2 µg/ml and 20 µg/ml. Even more preferably the concentration of TGFβ is between 0.2 µg/ml and 2 µg/ml. For example, the concentration of TGFβ is 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2.0 µg/ml.

In a still further embodiment the concentration of TGFβ is between 20 µg/ml and 125 µg/ml. For example, the concentration of TGFβ is 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, or 125 µg/ml.

The composition may also comprise one or more additional components, such as an osmotic agent, additional buffering agent, and preservative agent. Preferably the osmotic agent is mannitol or glucose. Preferably the mannitol is at a concentration between 2% and 10% w/w, even more preferably the mannitol is at a concentration between 4% and 5% w/w. For example, the mannitol is at a concentration of 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0% w/w.

Preferably the preservative agent is benzoic acid, methyl paraben, propyl paraben or benzyl alcohol.

The composition may also comprise hydrochloric acid, acetic acid or sodium hydroxide. Preferably the composition comprises water.

In a further embodiment, the composition is adapted for intra-vaginal administration.

In a second aspect the invention provides an article of manufacture comprising:

1. a vial, cartridge, or vaginal applicator; and
2. a composition according to the invention.

Preferably the article of manufacture is accompanied by, or labeled with, instructions that the article be used in the treatment of an infertility condition. For example the infertility condition may be recurrent miscarriage. Preferably the vial, cartridge, or vaginal applicator is sealed. Preferably the article of manufacture is accompanied by, or labeled with, instructions that the article be stored at 27°C or below, or between 2° and 8°C. Preferably the article of
manufacture is accompanied by, or labeled with, instructions that the composition is stable for at least 6 months, more preferably at least 12 months, when stored at temperatures between 2° and 27°C, or when stored at temperatures between 2° and 8°C, or when stored at room temperature.

In a third aspect the invention provides an article of manufacture comprising:
(1) a first vial or cartridge comprising a liquid composition comprising a buffering agent in an amount sufficient to maintain the pH of the composition above 3.7, and a zwitterion;
(2) a second vial or cartridge comprising a dry powder composition comprising a freeze-dried TGFβ; and
(3) instructions that the liquid composition is added to the dry power composition prior to use.

In a fourth aspect the invention provides a process for preparing a composition according to a first aspect of the invention, said process comprising the steps of adding a zwitterion and a pharmaceutically acceptable carrier to a substantially purified TGFβ.

In a fifth aspect the invention provides a process for preparing an article of manufacture, comprising sealing a composition according to the invention in a vial, cartridge or vaginal applicator, from which a therapeutically effective dose of the composition can be administered to a patient in need thereof. Preferably the process further comprises subjecting at least one component of the pharmaceutical composition to filtration, heat, or gamma irradiation sterilization. It will be appreciated that the TGFβ itself cannot be heat-sterilized; however, it can be subjected to gamma irradiation or filtration and then mixed with other heat-sterilized components of the composition, such as the gel polymer.

In a sixth aspect the invention provides a method of treating an infertility condition, comprising administering a composition according to the invention to a prospective mother. In one embodiment of this aspect of the invention the method comprises exposing a mucosal surface of said prospective mother to:
(1) a composition according to the invention; and
(2) semen or an MHC Class I antigen of a prospective father capable of eliciting a Th-1 response.

Preferably the infertility condition is implantation failure or a gestational disorder. Preferably, the implantation failure is recurrent implantation failure. More preferably the gestational disorder is recurrent miscarriage, pre-eclampsia
and intra-uterine growth retardation. Most preferably the gestational disorder is recurrent miscarriage.

In a further embodiment, the prospective mammalian mother lacks an immune tolerance to a paternal antigen and exhibits a Th-1 immune profile indicating a lack of said immune tolerance. Preferably the Th1 immune profile is indicated by a Th1 cell number higher than the Th2 cell number. Preferably the Th1 cell number and Th2 cell number profiles are derived from cervical brush samples, vaginal wash samples, or peripheral blood. In a preferred embodiment, the exposure is a multiple exposure. Alternatively the gestational disorder or implantation failure is caused by a failure of Treg cells to induce immune tolerance.

Preferably the composition is administered prior to, during, and/or after intercourse. For example the composition is administered over a period spanning at least one month before conception. Alternatively the composition is administered over a period spanning at least one week before conception. Preferably the composition is administered over a period spanning at least 4 days before conception. In a preferred embodiment the MHC Class I antigen is from sperm cells of the prospective father. Preferably the semen or an MHC Class I antigen of the prospective father is in the form of the prospective fathers ejaculate.

In one embodiment the mucosal surface is a genital mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or an oral mucosal surface. Preferably the mucosal surface is a genital mucosal surface. More preferably the composition is administered in a therapeutically effective amount.

In a seventh aspect the invention provides a method of reducing the adherence of TGFβ to a surface, said method comprising the addition of a zwitterion, as described above, to a composition comprising TGFβ. The composition may also optionally comprise a gel polymer, as described above.

In an eighth aspect the invention provides a method of reducing the precipitation of TGFβ in solution, said method comprising the addition of a zwitterion, as described above, to a composition comprising TGFβ.

In a ninth aspect the invention provides a method of characterising TGFβ in a composition, said method comprising evaluating the TGFβ by means of an analytical method, wherein the composition also comprises a zwitterion, as described above.
In one embodiment the analytical method is SDS-PAGE analysis or reversed phase HPLC. In another embodiment the analytical method is a bioassay, such as a mink lung epithelial cell growth inhibition assay. In one embodiment of this aspect, the composition is a composition according to the first aspect of the invention. The standard assay uses Mv1 Lu mink lung epithelial cells (CCL-64; American Type Culture Collection, Manassas, Va.)

In a tenth aspect the invention provides an article of manufacture comprising:
(1) a first vial or cartridge comprising a liquid composition comprising a buffering agent in an amount sufficient to maintain the pH of the composition at a specific pH and glycine;
(2) a second vial or cartridge comprising a dry powder composition comprising a freeze-dried TGFβ; and
(3) instructions that the liquid composition is added to the dry powder composition prior to use.

In an eleventh aspect the invention provides an article of manufacture comprising:
(1) a first vial or cartridge comprising a liquid composition comprising a buffering agent in an amount sufficient to maintain the pH of the composition above 3.7, and a TGFβ;
(2) a second vial or cartridge comprising a zwitterion, as described above; and
(3) instructions that the liquid composition is added to the gel polymer composition prior to use.

In the tenth and eleventh aspects of the invention, the pH ranges and concentrations of the components of the composition are as described in relation to the first aspect of the invention.

In all aspects of the invention the TGFβ is preferably a recombinant human TGFβ selected from the group consisting of TGFβ1, TGFβ2 and TGFβ3.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the results of a pregnancy outcome study. A single dose of TGFβ3 administered to female mice lacking immune tolerance to paternal antigen, either before attempted conception or after attempted conception, prevents miscarriage and has no detrimental affect on pregnancy.
DETAILED DESCRIPTION

In the claims of this application and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the words "comprise" or variations such as "comprises" or "comprising" are used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

As used herein, the term "substantially purified" refers to a TGFβ protein, functional analogue, derivative or biologically active fragment thereof, which has been removed from its natural environment, isolated or separated, and is at least 60% free, preferably 75% free, and most preferably 90% free from other components with which it is naturally associated.

A "zwitterion" is a dipolar ion which carries separate positively and negatively charged groups, and thus has no net charge. For example, amino acids have amino and carboxyl groups which are ionized at pHs in the physiological range.

The term "storage stable" as used herein with reference to compositions comprising TGFβ is taken to mean that the TGFβ maintains solubility and/or bioactivity for at least 6 months when stored at between 2° and 80°C, and preferably when stored at between 2° and 27°C. In a preferred embodiment the TGFβ maintains both solubility and bioactivity for at least 6 months when stored at between 2° and 80°C, and preferably when stored at between 2° and 27°C.

The term "pharmacologically inert", as used with reference to a compound means that the compound does not have any significant pharmacological activity, either alone or in the presence of other compounds. Thus its pharmacological activity is sufficiently low that it would be regarded as a pharmaceutically-acceptable carrier.

As used herein, the term "infertility condition" includes implantation failure and gestational disorders. Gestational disorders may include recurrent miscarriage, pre-eclampsia and intra-uterine growth retardation.

The term "TGFβ" as used herein refers to any member of the TGFβ superfamily, or to a biologically active fragment, functional analogue or derivative thereof. The TGFβ polypeptide used in the composition of the invention may be selected from the group consisting of TGFβ1, TGFβ2, TGFβ3, TGFβ4, TGFβ5 or activin (including activin A, activin B and activin AB). More preferably the TGFβ polypeptide is selected from the group consisting of TGFβ1, TGFβ2, TGFβ3 or activin (including activin A, activin B and activin AB).
Preferably the TGFβ polypeptide is human TGFβ; however, it will be clearly understood that TGFβ polypeptides from other species may be used. For example bovine TGFβ polypeptide may be used. Most preferably the TGFβ is recombinant human TGFβ.

Alternatively another member of the TGFβ superfamily may be used, including polypeptides selected from the group consisting of Mullerian inhibitory substances (MIS), bone morphogenetic proteins (BMP-2-7), inhibins, growth differentiation factors (GDF-1), dorsalin-1 (ds1-1) and Drosophila decapentaplegic gene product (DPP-C). The suitability of other members of the TGFβ superfamily for use in the composition can be assessed by no more than routine experimentation, for example using the mink lung epithelial cell growth inhibition assay. Preferably the TGFβ polypeptide member of the TGFβ family contains an intact cystine knot, such that the TGFβ family member is biologically active.

The TGFβ may be purified from tissue such as bone, platelets, or placenta. Alternatively, recombinant TGFβ may be used. Preparation of TGFβ by recombinant methods is well known in the art; see for example US Patent No. 5922846.

It will also be understood that various modifications may be made to the TGFβ. Such modified TGFβs may include substitution, deletion or addition mutants, and might include peptide fragments, which may be optionally incorporated into another protein to make a recombinant protein. Alternatively other polypeptide members of the TGFβ superfamily may also be used or used as a starting point for analogue production.

It is to be clearly understood that the present invention extends to biologically active fragments, functional analogues and derivatives of TGFβ, i.e. fragments, analogues or derivatives of TGFβ in which the wild-type TGFβ sequence contains additions, deletions or substitutions by other amino acids or amino acid analogues, but in which the biological activity of the TGFβ is retained. Methods for identifying, manufacturing and characterizing biologically active fragments, functional analogues or derivatives of TGFβ are well known to those of ordinary skill in the art, and can be used with no more than routine experimentation. Persons skilled in the art will be able to determine with a reasonable expectation of success which modifications can be made to the TGFβ fragment, analogue or derivative to conserve biological activity. In a preferred form, the fragment, functional analogue or derivative of TGFβ to be used will have substantially the same biological activity as native TGFβ, e.g.
binding to Type I or Type II TGFβ receptors and inhibiting the growth of Mink Lung cell line CCL64, or stimulating GM-CSF production by murine uterine epithelial cells \textit{in vitro}. Preferably the modification does not alter the tertiary structure of the cystine knot. Even more preferably the modification does not alter the TGFβ receptor binding site. In a preferred form the TGFβ fragment, functional analogue or derivative has at least 70% amino acid sequence homology with the native TGFβ amino acid sequence, or preferably at least 90%, more preferably 95%. Methods for assessing the amino acid sequence homology are well known in the art. For example, a suitable program for determining percentage sequence identity is \textit{BLAST 2.0 Sequence Comparison} (\textit{NIH}). Preferably, the limiting parameters imposed for such a task are the default settings for the program.

The invention also includes TGFβ in which the coding sequence for the polypeptide is fused in-frame to a polypeptide sequence which aids in expression of the fusion protein from a host cell. For example, a polypeptide leader sequence encoding a fragment of pig growth hormone. Other suitable fusion protein partner leader sequences are known in the art.

The three mammalian TGFβs (TGFβ1, TGFβ2 and TGFβ3) all possess three major activities: they inhibit proliferation of most cells, but can stimulate growth of some mesenchymal cells; they enhance the formation of extracellular matrix; exert immune inhibitory effects (Lawrence DA 1996 Transforming growth factor-beta: a general review. \textit{European Cytokine Networks} 7:363-374.) and exert immune tolerance induction properties (International Patent Application No. PCT/AU98/00149). The three mammalian TGFβs are involved in wound repair processes and starting inflammatory reactions and then in their resolution. The latter effects of the TGFβs derive in part from their chemotactic attraction of inflammatory cells and of fibroblasts (reviewed by Lawrence, 1996). The mammalian TGFβs have pleiotrophic and profound effects on the immune system and on hematologic malignances.

The activin subfamily comprises three isoforms of 28kDa polypeptides, namely activin A, activin B and activin AB. Activin binds to the constitutively active Ser/Thr kinase receptor activin type II (ActRII) or one of four isoforms of activin type NB (ActRIIB). Activin's primary role appears to be as a regulator of the pituitary gonadotropins, and in particular as a positive regulator of follicle stimulating hormone.

Pharmaceutical applications of TGFβ are widely described in the art; these include treatments for oral mucositis, angiogenesis, cancer, promoting
tissue repair of bone or cartilage, and prevention of scarring. However, no pharmaceutical formulations which contain a TGFβ have so far been approved by the United States Food and Drug Administration (FDA) or European Agency for the Evaluation of Medicinal Products (EMEA).

International Patent Application No. PCT/AU98/00149 describes a method of treating an infertility disorder with the administration of TGFβ wherein the disorder is characterized by a lack of immune tolerance by the prospective mother's immune system to paternal antigens. This technology is applicable to the treatment of conditions such as recurrent miscarriage, pre-eclampsia and intra-uterine growth retardation. The term "immune tolerance" is taken to mean inhibition of the potentially destructive cell-mediated immune response against specific conceptus antigens, and/or inhibition of synthesis of conceptus antigen-reactive immunoglobulin of complement-fixing isotypes (for example the ThV compartment of the immune response). Immune tolerance is antigen specific and is not generalized immunosuppression which is non-antigen specific. Additionally, PCT/AU98/00149 discloses that TGFβ, when administered to the female reproductive tract together with sperm or semen, can elicit tolerance towards male antigens, including paternal MHC class I antigens. This state of immune tolerance is evidenced by inhibition of Th1-type immune responses to paternal antigens, including delayed-type hypersensitivity (DTH) responses primed by a previous injection with sperm, production of complement-fixing isotypes of immunoglobulin specific for sperm, and cell-mediated immune rejection of tumor cells bearing the same MHC class I antigens as contained in the priming sperm inoculum.

Example 1 of PCT/AU98/00149 demonstrates that seminal TGFβ initiates the post mating inflammatory response in mice and humans. Example 2 of PCT/AU98/00149 demonstrates that seminal vesicle fluid modulates maternal reproductive performance and the maternal immune response to paternal antigens in mice. Example 3 of PCT/AU98/00149 demonstrates that the delivery of paternal antigens in combination with TGFβ to the female reproductive tract can generate systemic paternal antigen-specific tolerance, specifically by inhibiting the Th1 compartment of the immune response. Example 4 of PCT/AU98/00149 demonstrates that paternal antigen-specific immune deviation improves reproductive performance. The study confirmed that women exposed to semen (containing paternal antigen and natural TGFβ) around the time of thawed embryo transfer have a reduced risk of early embryonic loss compared to those instructed to abstain. Balb/cF1 female mice
also were immunised by intra-uterine infusion with CBA sperm in the presence
or absence of 10ng rTGFβi, and were mated naturally with CBA males 2
weeks later. Females were sacrificed on day 17 of pregnancy and the number
of total, viable and resorbing implantation sites, as well as fetal and placental
weights of viable conceptuses, were determined and showed that TGFβ
improved pregnancy outcome.

Studies reported in PCT/US94/02527 also demonstrate that the
presence of TGFβ in the female reproductive tract at the time of implantation
facilitates the production of fibronectin, a protein thought to assist implantation
by promoting adhesion of the embryo to the endometrial surface, and thus
increases the success rate of IVF procedures.

When the composition is adapted for topical or vaginal administration,
irritation is more likely to occur below pH 3.7 than above pH 3.7. Irritation is a
significant problem in the development of formulations adapted for
administration to the cervix or uterus via intra-vaginal delivery. Moreover, when
the composition is to be used for the treatment of an infertility condition the
composition should not be toxic to sperm or adversely effect conception. A
reduction of sperm viability is more likely to occur below pH 3.7 than above pH
3.7. Therefore for topical or vaginal administration, or for treatment of infertility,
a pH of above 3.7 is preferred.

If the liquid composition comprises a gel polymer, any hydrolysis of the
gel polymer during heat or radiation sterilization of the non-protein components
of the composition is undesirable, as this alters viscosity, thus affecting the
release profile of the TGFβ. Loss of viscosity of the gel polymer is more likely to
occur following heat sterilization when the pH of the pharmaceutical
composition is below 3.7. Therefore if the gel polymer is to be subjected to heat
sterilization, a pH of above 3.7 is preferred.

Preferably, the zwitterion present in the composition is at a concentration
of between 0.01% and 1% w/w, more preferably at a concentration of 0.15%
w/w.

In a preferred embodiment the composition is in liquid form, not in
freeze-dried form. The composition may be in gel form, comprising a gel
polymer. As used herein, the term "liquid" includes solutions and gel polymers.

In a preferred embodiment the composition comprises a gel polymer to
increase viscosity of the composition, thus aiding in delivery of the TGFβ at the
site of therapeutic action. Preferably the polymer is a gel-like substance suitable
for pharmaceutical use.
The inventors have found that the presence of a gel in the formulation reduces adherence of TGFβ to surfaces of containers and equipment. Adherence of TGFβ to surfaces is a problem for three reasons:

1. It reduces the overall dose delivered to the patient because the TGFβ is formulated at a low concentration and a large amount of the TGFβ will be lost by sticking to the container or delivery device;

2. It results in low yields during manufacture as the TGFβ will adhere to equipment and lines, thus increasing the complexity of manufacturing; and

3. Assaying the concentration of the TGFβ in the product is required for product release and will be difficult, because the TGFβ will adhere to vials and instruments, thus resulting in an inaccurate determination of TGFβ concentration.

In one specific embodiment the gel aids in the retention of the TGFβ at the site of delivery. Furthermore, gels have certain mucoadhesive properties and aid in delivery when administered to a mucosal surface, such as the cervical or uterine surface of a prospective human mother. In a preferred form, the gel polymer allows for release of the TGFβ at the site of delivery, thus making the TGFβ bioavailable. Suitable gel polymers have been described above.

In a preferred form, the composition is sterile, as described in the British Pharmacopoeia 2001.

In another preferred form, the composition has a low bioburden level, especially when adapted for topical delivery, including intra-vaginal delivery.

In one embodiment the composition has:

(a) Not more than a total of $10^2$ micro-organisms (aerobic bacteria or fungi) per gram, as tested using standard pour plate methods described in Appendix XVI B2 of the British Pharmacopoeia 2001.

(b) Not more than $10^1$ enterobacteria and certain other Gram-negative bacteria per gram, as tested using standard pour plate methods described in Appendix XVI B2 of the British Pharmacopoeia 2001.

(c) Absence of *Pseudomonas aeruginosa*, determined on 1g, as tested using standard pour plate methods described in Appendix XVI B2 of the British Pharmacopoeia 2001.

(d) Absence of *Staphylococcus aureus*, determined on 1g, as tested using standard pour plate methods described in Appendix XVI B2 of the British Pharmacopoeia 2001.
This low bioburden profile can be achieved by subjecting the composition to sterilization, as described above. Following sterilization, the composition is sealed in a pharmaceutically acceptable container such as a vial, sachet, cartridge or applicator. Preferably, if the non-protein components of the composition are heat sterilized, the TGFβ is sterilized separately by irradiation or microfiltration and added to the composition following heat sterilization.

The composition can comprise an osmolality agent, such as mannitol, lactose, glucose, glycerol, propylene glycol, sodium lactate or sodium citrate.-

Preferably, the osmolality agent is mannitol.

Preferably the osmolality agent is at a concentration of between 2% and 10% w/w. More preferably the osmolality agent is at a concentration of 4% or 5% w/w. If mannitol is used the concentration is preferably 4% or 5% w/w. If lactose is used the concentration is preferably 10% w/w. If glucose is used the concentration is preferably 5% w/w. If glycerol is used the concentration is preferably 2.5% w/w. If propylene glycol is used the concentration is preferably 2% w/w. If sodium lactate is used the concentration is preferably 4.5% w/w. If sodium citrate is used the concentration is preferably 3% w/w.

The composition may also comprise an additional buffering agent, such as acetic acid. The composition may comprise a salt such as magnesium hydroxide, provided that it does not cause precipitation of TGFβ. This may readily be assessed using routine methods.

The composition may also comprise a preservative agent, such as benzoic acid, methyl paraben, propyl paraben or benzyl alcohol. If benzoic acid or methyl paraben are used, the concentration of each agent is preferably between 0.01 and 1.0% w/w. Most preferably the concentration of benzoic acid is 0.1% w/w and the concentration of methyl paraben is 0.03% w/w. If benzyl alcohol is used, the concentration is preferably between 0.01 and 3% w/w. Most preferably the concentration is between 1.5% w/w and 2% w/w.

In an alternative embodiment the composition comprises a combination of preservative agents.

The composition can comprise hydrochloric acid, acetic acid or sodium hydroxide for pH adjustment.

The composition can comprise water to bring the difference in concentration to 100% w/w.

The invention further provides a vaginal applicator comprising the composition according to the invention. In a preferred form the applicator is
sealed such that the composition retains its low bioburden profile thus maintaining suitability for clinical use. In a preferred form the applicator is labeled with, or accompanied by, instructions for use of the device and composition for the treatment of recurrent miscarriage, pre-eclampsia, intra-uterine growth retardation or implantation failure. Preferably the instructions are for the treatment of recurrent miscarriage. Suitable vaginal applicators are well known in the art. For example a suitable intra-vaginal applicator for administering the pharmaceutical composition to the cervix can be purchased from Hueter Toledo Inc., Bellevue, Ohio, USA, which is manufactured using Huntsman P4C6N-041 polypropylene (for cap material), Huntsman P4C6N-041 polypropylene (for barrel material) and Santoprene 8281-55 rubber (for piston material).

The invention also comprises a pharmaceutically acceptable vial or container. In a preferred form the vial or container is sealed such that the composition retains its low bioburden profile thus maintaining suitability for clinical use. In a preferred form the vial or container is labeled with, or accompanied by, instructions for use of the device and composition for the treatment of recurrent miscarriage.

Preferably the vial or container is constructed out of plastic, or glass such as siliconised glass.

A number of assay methods (including quantification and qualification) can be used in the characterization of TGFβ in the composition. Such assays are used routinely for the testing of protein drug substances. Methods used in the quantification of TGFβ include reversed phase HPLC. Preferably the sample of TGFβ is compared with the profile of a reference standard of TGFβ to determine concentration. Furthermore, HPLC can be used for the characterisation of TGFβ by determining the presence of aggregates and degradation products. SDS-PAGE analysis can be used in the qualification of TGFβ stability by detecting the presence of aggregates and degradents.

The inventors have found that the presence of a gel in the composition inhibits the adherence of TGFβ to surfaces of containers, including containers and equipment lines used in testing the TGFβ, thus enhancing the accuracy of the assay method used for quantification and qualification. Therefore the loss of TGFβ to surfaces of containers and equipment is reduced in the presence of a gel. Preferably the gel used in the composition is a cellulose-based polymer, and more preferably the gel is hypromellose.
Preferably the TGFβ to be assayed is below a concentration of 100µg/ml and more preferably below 20µg/ml, even more preferably below 4µg/ml.

According to a fifth aspect the invention provides a method of treating an infertility condition, comprising the step of administration of the composition herein described to a subject in need of such treatment. The subject is preferably a mammal, more preferably a human.

Implantation failure may be caused by lack of fibronectin production by the conceptus.

The method according to a fifth aspect of the invention includes the treatment of a gestational disorder in a patient by inducing immune tolerance to a paternal antigen by the administration of the pharmaceutical composition herein described. The gestational disorder is preferably a disorder characterized by a lack of immune tolerance to paternal antigen and is not a disorder characterized by a lack of fibronectin production by the trophoblast.

Preferably the patient is a mammal. More preferably the patient is a human.

Methods used to treat such gestational disorders by the administration of TGFβ are described in International Patent Application No. PCT/AU98/00149, the entire contents of which are incorporated herein by this reference. The method comprises exposing a mucosal surface of a prospective mother, who lacks an immune tolerance to paternal antigen, to semen or MHC Class I antigen on the sperm of a prospective father capable of eliciting a Th-1 response, together with a substantially purified TGFβ.

Whilst a mucosal exposure may be preferred because it is likely to give rise to a transient tolerant immune reaction, it may also be feasible to provide for another route of exposure. Thus the pharmaceutical composition may be injected for systemic contact.

The mucosal surface can be a genital mucosal surface, a respiratory mucosal surface, a gastrointestinal mucosal surface or an oral mucosal surface. The mucosal surface is preferably the cervical mucosal surface.

Methods of identifying and selecting patients who have gestational disorders caused by a lack of immune tolerance are widely available. It is expected that the patient would have tested positive to a pregnancy test (hCG test), but then tested negative, thus indicating successful implantation and then pregnancy loss, and would have an immune profile indicating the presence of a Th1-dominant compartment, thus indicating lack of immune tolerance. Thus the patient to be treated has a dominant Th1 response to paternal antigen

Hence a diagnostic assay of interest is one that determines whether Th1 cell number or cell activity is increased in the prospective mother compared to the historical controls. Another assay of interest is one that determines whether Th2 cell numbers or activity is decreased compared to the historical control. Yet another assay of interest is one that determines whether Th1 cell numbers are higher in a patient when compared to Th2 cell numbers, or whether Th1 cell activity is higher in a patient when compared to Th2 cell activity, which would indicate the presence of a gestational disorder caused by lack of immune tolerance to paternal antigen. A number of known assays, for example, immunoassays or bioassays, can be used to make such determinations. For example interferon gamma, tumor necrosis factor alpha, and IL-1 and IL-2 are cytokine markers of Th1 cells. Thus assays for one or more such cell-specific markers can provide the basis to conclude a higher than normal Th1 status. As to Th2, the cytokines IL-4, IL-5, IL-6, IL-10 and IL-13 are known markers of that cell type. In a preferred embodiment, the TM cell is a tumor necrosis factor alpha-expressing CD3+/CD4+ T cell. In another preferred embodiment, the Th2 cell is an IL-4-expressing CD3+/CD8+ T cell. Suitable methods of determining Th1 levels to diagnose a patient with a gestational disorder caused by a lack of immune tolerance to paternal antigen are described in International Patent Application No. PCT/US2003/027204.

Thus, assays for one or more of such cell-specific markers can provide the basis to conclude a higher than normal Th2 status.

In a preferred form the patients who have the gestational disorders caused by a lack of immune tolerance do not have problems associated with lack of fibronectin production by trophoblasts.

The composition and/or the paternal antigen may be administered before or during attempted conception and after conception has been achieved.

It may be desirable to deliver the composition and the antigen together, for example, where the two are combined in a gel, or spray. It is also possible to
have a delay between the delivery of the pharmaceutical composition and the paternal antigen. Thus an alternative would be to deposit the antigen first perhaps as an ejaculate and then deliver the TGFβ as a pessary after intercourse.

Suitable paternal antigens include those that are particularly antigenic and prominent either on the sperm, or on the conceptus. The most likely candidates are MHC antigens, and more preferably MHC class I. The most efficient manner of presenting these antigens is in the form that they are naturally present - on any appropriate cell of the intended male parent which expresses them; such cells include sperm cells and leukocytes. The antigens may also be presented in biological fluids, such as seminal plasma, which is known to carry certain male antigens. This use of cells other than sperm cells will be pertinent where the sperm count of the prospective father is somewhat low. The use of cells other than sperm cells may be preferred where a non-genital route is used. Alternatively the antigens may be presented in purified or semi-purified form, which may optionally be presented on inert or adjuvant carriers; thus for example they may be presented in the carriers known as ISCOMS. It is additionally possible that the antigens may be encoded within sperm cells in the form of mRNA (or other nucleic acid), and this RNA message is then expressed by maternal genital tract cells. It may be that TGFβ therefore plays a role in promoting the events leading to presentation of paternal antigen to maternal lymphocytes through activating genital tract antigen presenting cells to take up and translate sperm mRNA.

The level of exposure to paternal antigens may vary. In a preferred form the exposure will be to the prospective mother's genital tract in the form of the prospective father's ejaculate, and the level of exposure will be determined by the cell count and antigenic density on the surface of such cells. Where cells are administered other than in the above manner, a similar number of cells may be used; however, the most effective manner may be determined empirically. It is thought that an exposure of leukocytes in the order of $10^7$ - $10^9$ cells might be the appropriate level of exposure to a mucosal surface.

The exposure is preferably a multiple exposure. The multiple exposure is preferably performed over a period of at least three months, with the mucosal surface being exposed to the pharmaceutical composition during each exposure to the prospective father's antigens. The multiple exposure can be performed over a period of at least one month before attempted conception. This period of time could however be somewhat reduced, and it may be
possible to achieve improvement with one exposure. However, as a minimum it is anticipated that exposure would be at least one week before conception is attempted. It may also be preferred that non-barrier contraceptive measures be taken prior to the planned conception, where the antigens are associated with sperm cells and these are administered to the genital tract, so that there is some certainty of a period of exposure to the prospective father's antigens before conception. This is particularly the case where the infertility condition is of the type where conception takes place, but miscarriage, spontaneous abortion or pre-eclampsia occurs after conception.

For example, suitable non-barrier contraceptive measures include hormonal contraceptives such as:

1. combined estrogen/progestin contraceptives administered by injection such as Cyclofem and Mesigyna which are administered once a month;
2. combined estrogen/progestin contraceptives administered orally such as Eugynon and Ovral which are administered daily;
3. progestin-only injectable contraceptives such as Depo-Provera and Noristerat which are administered every 3 months;
4. progestin-only oral contraceptives (also known as minipills) such as levonorgestrel (morning after pill), norgestrel which are taken daily;
5. progestin-only implants such as Norplant which are inserted once and can be effective for up to five years;
6. intrauterine devices (IUDs) such as Copper T 380A (copper releasing); or
7. progestin-releasing IUDs such as Progestasert.

Preferably the non-barrier method used does not inhibit the effectiveness of the TGFβ treatment. For example IUDs which are known to cause pelvic inflammatory diseases in some patients are preferably not used. Preferably contraceptive methods which have long-term residual effects on fertility following termination of use are not used. For example combined estrogen/progestin contraceptives administered by injection, progestin-only injectable contraceptives, and progestin-only implants, which are known to exhibit long lasting residual on fertility effects, are preferably not used. Most preferably, combined oral estrogen/progestin contraceptives and progestin-only oral contraceptives are used.

Suitable non-barrier contraceptive measures also include male contraceptives. For example suitable male contraceptives include methods
which suppress sperm production, such as gonadotropin-releasing hormone, or which inhibit the ability of the sperm to fertilise, such as Nifedipine.

Alternatively it may also be preferred that barrier contraceptive measures are used where the antigens are administered via mucosal surfaces other than to the genital tract such as the respiratory or gastrointestinal tract. For example the antigen may be presented as an oral, anal or nasal spray or gel.

Alternatively it might be desired to take the TGFβ and the surface antigen in a form that gives exposure to the small and perhaps large intestines, such as a gelatin capsule. Suitable barrier contraceptive measures include male and female condoms, diaphragms and spermicides.

The present invention may be used in conjunction with IVF treatment, whereby the transient tolerant immune response is elicited before transfer of the conceptus or gametes is attempted. It is expected, however, that where the gestational disorder is caused as a result of reduced TGFβ level in semen, or capacity to activate TGFβ, it is likely that IVF treatment may not be needed and that a 'natural' conception may be possible.

In accordance with this invention, the TGFβ is administered in therapeutically effective amounts. The term "therapeutically effective amount" as used herein means that amount necessary at least partly to attain the desired effect, i.e. the elicitation of tolerance towards male antigens. Such amounts will depend on the particular infertility condition being treated, the severity of the condition, and the characteristics of the individual subject, including age, physical conditions, size, weight and other concurrent treatments, and will be at the discretion of the attending physician. These factors are well known to those of ordinary skill in the art, and can be addressed with no more than routine experimentation. It is generally preferred that a minimum effective dose be determined according to sound medical judgment. It will be understood by those of ordinary skill in the art that a higher dose may be administered for medical, psychological or other reasons.

The level of TGFβ in the composition may be varied. The TGFβ will preferably be at a concentration of between 0.01 μg/ml and 125μg/ml, more preferably between 0.1 μg/ml and 100μg/ml. For example, the concentration may be between 0.2μg/ml and 20μg/ml, or between 0.2μg/ml and 2μg/ml. More preferably the concentration is 0.2μg/ml.

Alternatively, the concentration is between 0.2μg/ml and 20μg/ml, wherein the total dose is between 0.5μg and 60μg. More preferably a concentration of between 0.2μg/ml and 2μg/ml is used, wherein the total dose is
between 0.6 µg and 6 µg. More preferably the concentration is 0.2 µg/ml, wherein the total dose is 0.6 µg.

For example the concentration may be 0.5, 2.0, 4.0 or 20 µg/ml and administered to the patient in a 3 ml volume providing a total dose of 1.5, 6.0, 12 or 60 µg respectively.

Frequently used pharmaceutically-acceptable carriers or auxiliaries include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described in Remington's Pharmaceutical Sciences, 20th ed. Williams & Wilkins (2000) and The British National Formulary 43rd ed. (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2002; http://bnf.rhn.net), the contents of which are hereby incorporated by reference. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's the Pharmacological Basis of Therapeutics (7th ed., 1985).

The pharmaceutical compositions are preferably prepared and administered in dosage units. Solid dosage units include tablets, capsules and suppositories. For treatment of a subject, depending on activity of the compound, manner of administration, nature and severity of the disorder, age and body weight of the subject, different daily doses can be used. Under certain circumstances, however, higher or lower daily doses may be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dosage unit or several smaller dosage units, or by multiple administrations of subdivided doses at specific intervals.

The pharmaceutical compositions according to the invention may be administered locally or systemically in a therapeutically effective dose. Amounts effective for this use will, of course, depend on the severity of the disease and the weight and general state of the subject. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of the pharmaceutical composition, and animal models may be
used to determine effective dosages for treatment of the cytotoxic side effects. Various considerations are described, e.g., in Langer, Science, 249: 1527, (1990). Formulations for oral use may be in the form of hard gelatin capsules, in which the active ingredient is 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, in which the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

A sterile injectable preparation may also a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanol. Among the acceptable vehicles and solvents which may be employed are water, Ringer’s solution, and isotonic sodium chloride solution. In addition, 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. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

TGFβ may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines.

The amount of active ingredient which may be combined with the carrier materials to produce a single dosage will vary, depending upon the host to be treated and the particular mode of administration.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease or condition undergoing therapy.

The compounds of the invention may additionally be combined with other compounds to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically-active agents, as long as the combination does not eliminate the activity of the TGFβ.

In a preferred form the composition is administered to the reproductive tract of a patient suffering a gestational disorder caused by lack of immune tolerance to paternal antigen, using a vaginal applicator. Alternatively the composition is delivered by oral administration (including buccal patch), topical
application, topical administration to a mucosal surface, subcutaneous application, or intramuscular delivery.

Abbreviations used herein are as follows;

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>rp-HPLC</td>
<td>Reverse-phase high performance liquid chromatography</td>
</tr>
<tr>
<td>GTR</td>
<td>GroPep technical report</td>
</tr>
<tr>
<td>DAD</td>
<td>Development analytical document</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra-acetic acid</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>Hypromellose</td>
<td>Hydroxypropylmethylcellulose (also known as HPMC)</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>MQ</td>
<td>Milli Q</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per unit volume</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per unit volume</td>
</tr>
</tbody>
</table>

The present invention will now be more fully described with reference to the accompanying non-limiting examples. It should be understood that the following description is illustrative only, and should not be taken in any way as a restriction on the generality of the invention.

**EXAMPLE 1. Manufacture and testing of four pharmaceutical formulations comprising TGFβ3**

Four different hypromellose gel formulations of TGF β3, designated Prototypes G, H, I, and J, were manufactured with the aim of identifying potential stable pharmaceutical formulations of the protein. All candidate formulations had a pH within the range of 3.3-3.7.

Four hypromellose gel vehicles were manufactured using the "hot-cold" method. Gels were packed in bulk, then sterilized by autoclaving at 121 °C for 15 minutes. TGF β3 was added to the sterile gels to produce gel formulations of 125 µg/mL TGF β3, which were then packed into 10 ml glass vials.

The four gel vehicles were prepared as follows.

Prototype G: Glycine buffer was prepared by combining 50g mannitol, 1.5g glycine, 1g benzoic acid, 550g purified water and 1.6mL of 10% hydrochloric acid solution. This solution was chilled to approximately 4°C.
Hypromellose 4000 was dispersed in 382g hot purified water in a 1L beaker. The cold buffer was added to the hot slurry of hypromellose while stirring to form the gel.

Prototype H: Acetic acid buffer was prepared by combining 40g mannitol, 710g purified water and 2.286mL glacial acetic acid and adjusting to pH 3.5 with sodium hydroxide. This solution was chilled to approximately 4°C. 19g Hypromellose 4000 was dispersed in 229g of hot purified water in a 1L beaker. The cold buffer was added to the hot slurry of hypromellose while stirring to form the gel.

Prototype I: Glycine buffer was prepared by combining 40g mannitol, 1.5g glycine, 0.7g methyl paraben, 0.3g propyl paraben, 708g purified water and 1.6mL of 10% hydrochloric acid solution. This solution was chilled to approximately 4°C. 19g Hypromellose 4000 was dispersed in 229g hot purified water in a 1L beaker. The cold buffer was added to the hot slurry of hypromellose while stirring to form the gel.

Prototype J: Glycine buffer was prepared by combining 40g mannitol, 1.5g glycine, 15mL benzyl alcohol, 695g purified water and 1.6mL of 10% hydrochloric acid solution. This solution was chilled to approximately 4°C. 18g Hypromellose 4000 was dispersed in 229g hot purified water in a 1L beaker. The cold buffer was added to the hot slurry of hypromellose while stirring to form the gel.

For each of the gel vehicles, 100mL glass vials were filled with 80g gel per vial, then sealed using bungs and crimp seals. Vials were sterilized by autoclaving at 121°C for 15 minutes.

TGFβ3 gel formulations were prepared from each of the gel vehicles by weighing approximately 470mL of the gel into a 250mL bottle. TGFβ3 drug substance solution (recombinant TGFβ3 stored in 20% EtOH / 20 mM acetic acid solution at a protein concentration of 9.1 mg/mL) was added to produce a final TGFβ3 concentration of 125μg/mL. The solution was mixed by slowly rotating the bottle for 30 minutes. The gel was packed into 10mL glass vials, at approximately 5g per vial. Vials were stored at 2-8°C, protected from light.

The TGFβ3 gel formulations were assessed for specific parameters using methods summarised in Table 1.
Table 1. Methods used for the assessment of TGFβ3 gel formulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance (clarity, colour, homogeneous consistency)</td>
<td>Visual inspection</td>
</tr>
<tr>
<td>pH</td>
<td>Potentiometry</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Brookfield Dial Viscometer</td>
</tr>
<tr>
<td>Osmolality</td>
<td>Freezing point depression (using a Hermann Roebling Automatic Micro-Osmometer)</td>
</tr>
<tr>
<td>Presence of TGFβ3 covalent aggregates and monomer</td>
<td>Reversed phase HPLC (to desalt samples) followed by polyacrylamide gel electrophoresis (see also below)</td>
</tr>
<tr>
<td>TGFβ3 concentration</td>
<td>HPLC on a Vydac C4 300Å, 5μm, 4.6 x 250 mm reversed phase column (Grace Vydac, Hesperia, CA, USA) at 40°C using a 20-80% acetonitrile gradient with 0.08% trifluoroacetic acid over 14 minutes at 1 ml/minute and UV detection at 215nm</td>
</tr>
</tbody>
</table>

Gel formulations were assayed for TGFβ3 covalent aggregates and monomer as follows. Gel samples were desalted by HPLC on a Vydac C4 5μm 300A, 4.6 x 250 mm reversed phase column (Grace Vydac, Hesperia, CA, USA), at 4°C using a 35-100% acetonitrile gradient with 0.08% trifluoroacetic acid over 14 minutes at 1ml/minute. Gel formulations were diluted 1 part in 6 in 0.1% trifluoroacetic acid, then a diluted gel sample containing 10μg of TGFβ3 was loaded and the 2.7ml eluate fraction from 6.9-9.6 minutes was collected. 10mg mannitol was added to stabilize the protein during lyophilization. This solution was dried to a white pellet under vacuum at room temperature. The pellet was reconstituted in 40μL of 1x NuPAGE® LDS Sample Buffer. 2μL of the reconstituted sample was withdrawn and diluted with 30μL of 1x Sample Buffer to allow protein content in the sample to be determined from a standard curve. The remaining 38μL (nominal 9.5μg) of the test sample and 32μL (nominal 0.5μg) of the diluted test sample were loaded onto a 10 x 1mm well NuPAGE® 4-12% Bis-Tris Pre-Cast gel (Invitrogen, San Diego, CA, USA). 10μg Reference Standard was prepared in an identical manner to the test sample by dilution in 1/6 gel then desalting as described above. The
reconstituted Reference Standard was then loaded on to the gel at nominal loads of 9.5 µg and 0.5 µg. These reference samples were used to determine the formation of aggregates and monomer in the Reference Standard due to the desalting procedure. Undesalted Reference Standard samples were also prepared in Sample Buffer and loaded on to the gel at 0.1, 0.3, 0.5 and 0.8 µg to allow quantitation of bands from a standard curve over this range. All test and reference samples were heated for 10 minutes at 70°C before loading. One lane was used for SeeBlue® molecular weight markers (Invitrogen, San Diego, CA, USA).

Gels were run in an Xcell II™ Mini-Cell with a BioRad PowerPac 300 at 200 V for 35 minutes in NuPAGE® MES SDS running buffer (Invitrogen, San Diego, CA, USA). Gels were fixed for 15 minutes in 50% methanol, 7% acetic acid then rinsed (3 x 5 minute rinses in ultrapure water) before staining with GelCode® Blue Stain Reagent (Pierce, Rockford, IL, USA) overnight. Gels were destained for 2 hours using several changes of ultrapure water. Gels were analysed using a Kodak EDAS 120 Digital Camera with Kodak™ Digital Science 1D Image Analysis v3.0 software.

The protein content of test sample bands was determined from band intensities compared to a standard curve on each gel, derived from band intensities of Reference Standard samples over the range of 0.1-0.8 µg. Aggregate content in test samples was corrected for the background content of aggregates in desalted Reference Standard by subtracting the impurity content in Reference Standard from the impurity content in test samples. Impurities are expressed as a percentage of the actual total protein content in the sample after HPLC desalting which is determined from quantitation of protein in the test sample loaded at a nominal 0.8 µg against the standard curve. The quantitation limit for the assay was 1% (0.1 µg) and detection limit was 0.5% (0.05 µg).

Results of release testing of TGFβ3 gel formulations are shown in Table 2.
Table 2. Results of testing TGFβ3 gel formulations immediately after their preparation

<table>
<thead>
<tr>
<th>Test</th>
<th>Prototype G</th>
<th>Prototype H</th>
<th>Prototype I</th>
<th>Prototype J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
</tr>
<tr>
<td>pH</td>
<td>3.33</td>
<td>3.52</td>
<td>3.63</td>
<td>3.51</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1200 mPa.s</td>
<td>1420 mPa.s</td>
<td>1380 mPa.s</td>
<td>1260 mPa.s</td>
</tr>
<tr>
<td>Osmolality</td>
<td>400 mOsmol/kg</td>
<td>340 mOsmol/kg</td>
<td>330 mOsmol/kg</td>
<td>480 mOsmol/kg</td>
</tr>
<tr>
<td>PAGE*</td>
<td>Not determined (assay failed)</td>
<td>Aggregates 0% Monomer &lt; 1%</td>
<td>Aggregates 0% Monomer &lt; 1%</td>
<td>Aggregates 0% Monomer &lt; 1%</td>
</tr>
<tr>
<td>TGFβ3 concentration (% of nominal)</td>
<td>95.7</td>
<td>92.1</td>
<td>94.8</td>
<td>95.7</td>
</tr>
<tr>
<td>Bioactivity**</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
</tr>
</tbody>
</table>

Calculated from the aggregates in desalted test sample minus aggregates in desalted reference standard sample.

*IC50 and maximum inhibitory response compared to National Institute for Biological Standards and Control (NIBSC) TGFβ3 reference standard.

All four formulations were acceptable with respect to physical properties, chemical tests and biological activity. All had concentrations of TGFβ3 within 10% of the nominal concentration. No aggregates were detected in any of the formulations. A small amount of monomer was detected in all formulations as a result of the presence of 0.5-1% monomer in the drug substance. TGFβ3 bioactivity recovered from all formulations had equivalent potency to the biological activity of NIBSC TGFβ3 reference standard. These candidate formulations were stored at 4°C and stability was monitored over 12 months.
EXAMPLE 2. Stability of TGFβ3 gel formulations

The stability of TGFβ3 in the gel formulations prepared in Example 1 was monitored over 12 months while stored at 4°C. Formulations were assessed for appearance, pH, viscosity, osmolality, turbidity indicating presence of insoluble aggregates, presence of monomer and covalent aggregates of TGFβ3, TGFβ3 concentration and TGFβ3 bioactivity after 6 and 12 months, using the methods described above.

The results of testing TGFβ3 gel formulations G, H, I and J after storage at 4°C for 6 and 12 months are shown in Tables 3, 4, 5 and 6.

Table 3. Results of testing prototype G after storage at 4°C for 6 and 12 months.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time zero</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
</tr>
<tr>
<td>pH</td>
<td>3.33</td>
<td>3.27</td>
<td>3.36</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1200 mPa.s</td>
<td>1230 mPa.s</td>
<td>1250 mPa.s</td>
</tr>
<tr>
<td>Osmolality</td>
<td>400 mOsmol/kg</td>
<td>400 mOsmol/kg</td>
<td>410 mOsmol/kg</td>
</tr>
<tr>
<td>Absorbance at 350 nm*</td>
<td>Not determined</td>
<td>0.0034 AU</td>
<td>0.0265 AU</td>
</tr>
<tr>
<td>PAGE**</td>
<td>Not determined</td>
<td>Aggregates &lt;1%</td>
<td>Aggregates 0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monomer &lt;0.5%</td>
<td>Monomer &lt;0.5%</td>
</tr>
<tr>
<td>TGFβ3 concentration (% of nominal)</td>
<td>95.7</td>
<td>92.5</td>
<td>95.2</td>
</tr>
<tr>
<td>Bioactivity***</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
</tr>
</tbody>
</table>

*absorbance of TGF-β3 gel minus absorbance of gel vehicle alone

**calculated from the aggregates in desalted test sample minus aggregates in desalted reference standard sample.

***IC50 and maximum inhibitory response compared to NIBSC TGFβ3 reference standard.
Table 4. Results of testing prototype H after storage at 4°C for 6 and 12 months.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time zero</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, colourless gel, homogeneous</td>
<td>Clear, colourless gel, homogeneous</td>
<td>Clear, colourless gel, homogeneous</td>
</tr>
<tr>
<td></td>
<td>consistency</td>
<td>consistency</td>
<td>consistency</td>
</tr>
<tr>
<td>pH</td>
<td>3.52</td>
<td>3.50</td>
<td>3.56</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1440 mPa.s</td>
<td>1440 mPa.s</td>
<td>1380 mPa.s</td>
</tr>
<tr>
<td>Osmolality</td>
<td>340 mOsmol/kg</td>
<td>340 mOsmol/kg</td>
<td>340 mOsmol/kg</td>
</tr>
<tr>
<td>Absorbance at 350 nm*</td>
<td>Not determined</td>
<td>0.0102 AU</td>
<td>0.0028 AU</td>
</tr>
<tr>
<td>PAGE**</td>
<td>Aggregates 0%</td>
<td>Aggregates 1.1%</td>
<td>Aggregates &lt;1%</td>
</tr>
<tr>
<td></td>
<td>Monomer &lt;1%</td>
<td>Monomer &lt;0.5%</td>
<td>Monomer &lt;0.5%</td>
</tr>
<tr>
<td>TGFβ3 concentration</td>
<td>92.1</td>
<td>89.9</td>
<td>90.2</td>
</tr>
<tr>
<td>(% of nominal)</td>
<td>Equivalent to NIBSC TGFβ3 reference</td>
<td>Equivalent to NIBSC TGFβ3</td>
<td>Equivalent to NIBSC TGFβ3</td>
</tr>
<tr>
<td>Bioactivity***</td>
<td>Equivalent to NIBSC TGFβ3 reference</td>
<td>Equivalent to NIBSC TGFβ3</td>
<td>Equivalent to NIBSC TGFβ3</td>
</tr>
</tbody>
</table>

*absorbance of TGF-β3 gel minus absorbance of gel vehicle alone

**calculated from the aggregates in desalted test sample minus aggregates in desalted reference standard sample.

***IC50 and maximum inhibitory response compared to NIBSC TGFβ3 reference standard.
Table 5. Results of testing prototype I after storage at 4°C for 6 and 12 months.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time zero</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
</tr>
<tr>
<td>pH</td>
<td>3.63</td>
<td>3.56</td>
<td>3.65</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1390 mPa.s</td>
<td>1390 mPa.s</td>
<td>1400 mPa.s</td>
</tr>
<tr>
<td>Osmolality</td>
<td>330 mOsmol/kg</td>
<td>340 mOsmol/kg</td>
<td>350 mOsmol/kg</td>
</tr>
<tr>
<td>Absorbance at 350 nm*</td>
<td>Not determined</td>
<td>0.0068 AU</td>
<td>0.0096 AU</td>
</tr>
<tr>
<td>PAGE**</td>
<td>Aggregates 0% Monomer &lt;1%</td>
<td>Aggregates 0% Monomer 3%</td>
<td>Aggregates 0% Monomer &lt;0.5%</td>
</tr>
<tr>
<td>TGFβ3 concentration (%) of nominal</td>
<td>94.8</td>
<td>93.1</td>
<td>96.4</td>
</tr>
<tr>
<td>Bioactivity***</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
</tr>
</tbody>
</table>

*absorbance of TGF-β3 gel minus absorbance of gel vehicle alone

**calculated from the aggregates in desalted test sample minus aggregates in desalted reference standard sample.

***IC50 and maximum inhibitory response compared to NIBSC TGFβ3 reference standard.
Table 6. Results of testing prototype J after storage at 4°C for 6 and 12 months.

<table>
<thead>
<tr>
<th>Test</th>
<th>Time zero</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
<td>Clear, colourless gel, homogeneous consistency</td>
</tr>
<tr>
<td>pH</td>
<td>3.51</td>
<td>3.42</td>
<td>3.51</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1290 mPa.s</td>
<td>1290 mPa.s</td>
<td>1270 mPa.s</td>
</tr>
<tr>
<td>Osmolality</td>
<td>480 mOsmol/kg</td>
<td>490 mOsmol/kg</td>
<td>480 mOsmol/kg</td>
</tr>
<tr>
<td>Absorbance at 350 nm*</td>
<td>Not determined</td>
<td>0.0000 AU</td>
<td>0.0066 AU</td>
</tr>
<tr>
<td>PAGE**</td>
<td>Aggregates 0% Monomer &lt;0.5%</td>
<td>Aggregates 0% Monomer &lt;1%</td>
<td>Aggregates 0% Monomer &lt;0.5%</td>
</tr>
<tr>
<td>TGFβ3 concentration (% of nominal)</td>
<td>95.7</td>
<td>94.1</td>
<td>92.2</td>
</tr>
<tr>
<td>Bioactivity***</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
<td>Equivalent to NIBSC TGFβ3 reference standard</td>
</tr>
</tbody>
</table>

* absorbance of TGF-β3 gel minus absorbance of gel vehicle alone
** calculated from the aggregates in desalted test sample minus aggregates in desalted reference standard sample.
*** IC50 and maximum inhibitory response compared to NIBSC TGFβ3 reference standard.

This 12 month stability study of four candidate gel formulations of TGFβ3 showed that all four formulations were physically and chemically stable and retained biological activity throughout the storage period at 4°C. The concentration of TGFβ3 remained within 10% of nominal; aggregates remained less than 2%, and monomer remained less than 1% for all formulations, except the 6 month test of prototype I, which was 3% but when tested at 12 months was <0.5%. The TGFβ3 biological activity of all four formulations remained equivalent to the bioactivity of NIBSC reference standard at all testing time points throughout the 12 month stability study.
EXAMPLE 3. Release of TGFβ from hypromellose.

The following experiment was performed to show that TGF-β is released from a hypromellose gel formulation.

TGF-β3 20µg/mL gel was prepared by mixing TGF-β3 in hypromellose gel (hypromellose 1.8%, mannitol 3.5%, glycine 0.15%, hydrochloric acid to pH 3.5, in water; autoclaved at 121°C for 15 minutes; viscosity 870 mPa.s). 2.5 ml TGF-β3 gel was placed in a 30 mm Millicell® insert with 0.4 µm hydrophilic polycarbonate membrane (Millipore, Billerica, MA, USA). The Millicell® donor well was suspended in 6mL of solution containing 5% mannitol (for isotonicity with the donor phase), 10% hypromellose gel (as a carrier to reduce adsorptive losses of TGF-β3) in 0.1% acetic acid in a receiver cell (6-well Millicell® culture plate). The receiver phase was stirred continuously for 18 hours, then the unit was disassembled and TGF-β3 content was measured in the donor phase, receiver phase and in 1 ml wash solution of 25% acetonitrile, 0.08% trifluoroacetic acid in water used to recover TGF-β3 from the membrane. TGF-β3 concentration was measured using HPLC on a Vydac C4 300 A, 5 µm, 4.6x250mm reversed phase column, at 40°C using a 20-80% acetonitrile gradient with 0.08% trifluoroacetic acid over 14 minutes at 1mL/minute and UV detection at 215nm.

54% of the initial TGF-β3 loaded into the donor cell remained in the donor phase after 18 hours. 14% was present in the receiver phase, and 15% was recovered from the membrane. There was an apparent loss of 17% of the initial amount of TGF-β3 loaded into the donor cell, which was probably due to adsorption on to the polystyrene surfaces of the device.

This experiment demonstrates that TGF-β3 is passively released from hypromellose gel in vitro and suggests that it would be bioavailable when administered in vivo.

EXAMPLE 4. Glycine as a stabiliser for TGFB3 at a pH of between 5.0 and 5.5

This study outlines the stabilising effect of glycine on TGFβ3 when exposed to pH conditions of between 5.0 and 5.5. It was observed that the presence of glycine as a component of the formulation prevented TGFβ3 from aggregating, even at pHs above pH 3.8, where TGFβ3 aggregation is known to occur (Pellaud J et al., J. Biol. Chem. (1999) 274; 7699-7704).
The experiments reported in this example showed that the recoveries of soluble TGFβ3 were improved in gel formulations containing glycine compared with formulations without glycine.

5 *Instrument and Conditions:*

<table>
<thead>
<tr>
<th>Instrument:</th>
<th>Shimadzu HPLC system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column:</td>
<td>Phenomenex ® Jupiter 5u C5 300A, 250 x4.60 mm, Part 00G-4052-E0</td>
</tr>
<tr>
<td>Column Temperature:</td>
<td>4°C</td>
</tr>
<tr>
<td>Buffer A:</td>
<td>0.1% TFA in Milli-Q water</td>
</tr>
<tr>
<td>Buffer B:</td>
<td>0.08% TFA in 80% Acetonitrile</td>
</tr>
<tr>
<td>Column wash:</td>
<td>50% Acetonitrile in Milli-Q water</td>
</tr>
<tr>
<td>Flow:</td>
<td>1mL/min</td>
</tr>
<tr>
<td>Detection wavelength:</td>
<td>215nm</td>
</tr>
<tr>
<td>Run time:</td>
<td>25mins</td>
</tr>
</tbody>
</table>

**Gradient Program:**

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow</th>
<th>Buffer B (%)</th>
<th>Buffer A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1.0</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>14.00</td>
<td>1.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>17.00</td>
<td>1.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>17.00</td>
<td>1.0</td>
<td>25.0</td>
<td>75.0</td>
</tr>
<tr>
<td>25.00</td>
<td>1.0</td>
<td>25.0</td>
<td>75.0</td>
</tr>
</tbody>
</table>

*Calculation for percentage recovery of TGFβ3 in supernatant:*

The percentage recovery was calculated as shown below:

\[
\% \text{ Recovery in supernatant} = \frac{\text{Peak area of supernatant} \times \text{theoretical load} \times 100}{\text{Peak area of "903 reference standard" for } x \mu g}
\]

25 **Part A:**

The acetate gel was manufactured using standard methods in the art, and pH was adjusted to 5.5. The gel contained the following components, 4% w/w mannitol, 0.22% w/w acetic acid, 0.17% v/w 1M sodium hydroxide, 1.25% w/w hypromellose 4000 cP grade was used throughout example 4) and MQ.
water made up to final volume, pH 3.45. The pH of the gel was further adjusted to pH 5.5 using NaOH.

The glycine buffer contained 0.15% w/w glycine, 4% w/w mannitol and hydrochloric acid pH 4.97.

Acetic acid and mannitol were dissolved in water and pH was adjusted to 3.45 using NaOH and the solution was then refrigerated. Hot water was added to hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel. The gel was further pH adjusted to pH 5.5 using NaOH for this experiment.

20 µg/ml of TGFβ3 formulated in an acetate-based gel at pH 5.0, was diluted 1 in 6 with a glycine-based buffer at pH 5.0. Samples were centrifuged for 8 minutes at 17320G and the supernatant analysed by rp-HPLC. The results are shown in Table 7.

Table 7: Percentage recovery of TGFβ3 in supernatant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Recovery in supernatant (of expected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>39.6</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>36.9</td>
</tr>
<tr>
<td>Average</td>
<td>38.3</td>
</tr>
</tbody>
</table>

The percentage recoveries for TGFβ3 in the supernatant of diluted acetate gel at about pH 5 were low (38.3%) as expected due to TGFβ3 aggregate formation at >pH 3.8 (Pellaud J et al., J. Biol. Chem. (1999) 274; 7699-7704).

Part B:

The glycine gel was manufactured according to standard methods in the art, and pH was adjusted to 5.0 (unadjusted pH 3.5).

The gel contained the following components: 4% w/w mannitol, 0.15% w/w glycine, 0.17% v/w 10% HCl, 1.9% w/w hypromellose and MQ water, pH 3.5. The gel was further pH adjusted to pH 5.0 using NaOH for this experiment. 2% v/v Benzyl alcohol was also added as a preservative. The
glycine buffer contained 0.15% glycine, 4% w/w mannitol and hydrochloric acid pH 4.97.

Glycine and mannitol were dissolved in water and pH was adjusted to 3.5 using HCl, and the solution was then refrigerated. Hot water was added to hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel solvent. The pH of the gel was further adjusted to 5.0 using NaOH.

20µg/mL of TGFβ3 was formulated in a glycine-based gel and diluted 1 in 6 using a glycine-based buffer at pH 5.0. All samples were centrifuged for 8 minutes at 17320g and the supernatant was analysed by rp-HPLC. The results are shown in Table 8.

Table 8: Percentage recovery of TGFβ3 in supernatant after centrifugation for 5-30 minutes.

<table>
<thead>
<tr>
<th>Duration of centrifugation at 17320g</th>
<th>% recovery in Supernatant (mean, 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min (pH5.0)</td>
<td>98.5</td>
</tr>
<tr>
<td>10 min (pH5.0)</td>
<td>97.1</td>
</tr>
<tr>
<td>20 min (pH5.0)</td>
<td>95.9</td>
</tr>
<tr>
<td>30 min (pH5.0)</td>
<td>95.9</td>
</tr>
<tr>
<td>30 min (pH3.4) control</td>
<td>92.4</td>
</tr>
</tbody>
</table>

The percentage recoveries in the supernatant were surprisingly high compared with those of observed with acetate gel. The only difference was the use of glycine as a buffering agent in the gel instead of an acetate salt. The presence of glycine in the gel had a stabilising effect on TGFβ3 even at pH 5.0, where aggregation of TGFβ3 is known to occur. Mass balance analysis confirmed that the remainder of the TGFβ3 was present in the pellet of the sample and not lost to the vessel surface.

Part C:

The aim of this part of the study was to confirm the observations in Parts A and B. Various formulations of TGFβ3 drug product at 20µg/mL were diluted
1 in 6 in different combinations of buffers. Sample preparation for rp-HPLC analysis was as described in Parts A and B.

The acetate gel was manufactured according to methods standard in the art and pH was adjusted to 5.5 using NaOH. The gel contained the following components, 4% w/w mannitol, 0.22% w/w acetic acid, 0.17% v/w 1M sodium hydroxide, 1.25% w/w hypromellose and MQ water, pH3.45. The pH of the gel was further adjusted to 5.5 using NaOH for this experiment. 2% v/v Benzyl alcohol was also added to one set of replicates. The glycine gel was manufactured according to methods standard in the art and pH was adjusted to 5.0.

The glycine gel contained the following components: 4% w/w mannitol, 0.15% w/w glycine, 0.017% v/w HCl, 1.9% w/w hypromellose and MQ water, pH 3.5. The gel pH was adjusted to 5.0 using NaOH for use in this experiment. 2% v/v Benzyl alcohol was also added. The glycine buffer contained 0.15% w/w glycine, 4% w/w mannitol and hydrochloric acid pH 4.97.

Acetic acid and mannitol were dissolved in water, the pH was adjusted to 3.45 using NaOH, and the solution was then refrigerated. Hot water was added to the hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel solvent. The pH was further adjusted to 5.5 and 2% v/v benzyl alcohol was also added to one set of replicates.

Glycine and mannitol were dissolved in water, the pH adjusted to 3.5 using HCl, and the solution was then refrigerated. Hot water was added to the hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel solvent. NaOH was added to achieve a final pH of 5.0 for this experiment. The results are shown in Table 9.
Table 9: Percentage recovery in the supernatant of the various different formulations of TGFβ3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% recovery in supernatant (mean, n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate gel pH 5.5 + glycine buffer pH 4.97</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>39.0</td>
</tr>
<tr>
<td>Acetate gel pH 5.5 (+ 2% benzyl alcohol) + glycine buffer pH 4.97 (final pH ~ 5.0 by pH strip)</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>54.9</td>
</tr>
<tr>
<td>Glycine gel pH 5.0 (+ 2% benzyl alcohol) + glycine buffer pH 5.0 (final pH ~ 4.5 by pH strip)</td>
<td>101.6</td>
</tr>
<tr>
<td></td>
<td>97.6</td>
</tr>
</tbody>
</table>

- Results are of duplicate samples

The percentage recoveries for the diluted acetate gel drug product samples were around 37% (as seen in Part A) and 100% for the glycine gel drug product samples at a similar pH. These results confirm that TGFβ3 formulated in glycine-buffered gel was more stable than TGFβ3 formulated in acetate-buffered gel.

Also note that even though glycine buffer was used to dilute the TGFβ3 acetate based gel, the recovery was still low. It seems therefore that glycine would have to be added to the gel in order for glycine to stabilise TGFβ3; however, Part E below shows that glycine can have a stabilizing effect without requiring the presence of gel.

**Part D:**

It was noted in Part C that the final pH of the glycine gel after dilution was about pH 4.5 whereas the acetate gel after dilution was about pH 5.0. The aim of this part of the study was to determine whether the small differences in the final pH of the diluted gels caused the difference in recoveries of TGFβ3 in the supernatant between glycine and acetate formulations, or whether the difference was due to a residual amount of acetate when samples were diluted for analysis.
The glycine gel was manufactured according to methods standard in the art, and pH was adjusted to 5.2 and 5.5 in this experiment. The gel contained the following components, 4% w/w mannitol, 0.15% w/w glycine, 0.017% v/w HCl, 1.9% w/w hypromellose and MQ water, pH 3.5. The gel was adjusted to 5.2 and 5.5 using NaOH. 2% v/v Benzyl alcohol was also added. The glycine buffer contained 0.15% glycine, 4% w/w mannitol and hydrochloric acid pH 4.97.

Glycine and mannitol were dissolved in water, pH was adjusted to 3.5 using HCl and the solution was then refrigerated. Hot water was added to the hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel solvent. NaOH was added to achieve a final pH of 5.2 or 5.5.

TGFβ3 drug product, at 20µg/mL, was formulated in glycine gel at pH 5.2 and 5.5 then diluted 1 in 6, centrifuged for 8 minutes at 17320 g and supernatant analysed by HPLC. The results are shown in Table 10.

Table 10: The difference in recovery of TGFβ in the supernatant with the two different formulations of TGFβ3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% recovery in supernatant (mean, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine gel pH 5.2 + glycine buffer pH 4.79 (final pH 5.2)</td>
<td>89.3</td>
</tr>
<tr>
<td>Glycine gel pH 5.5+ glycine buffer pH 4.79 (final pH 5.3)</td>
<td>89.0</td>
</tr>
</tbody>
</table>

Raising the pH of the gel from 4.5 to 5.2 and 5.5 had little impact on the recovery of soluble TGFβ3 in the supernatant. These results confirm that glycine was acting as a stabiliser for TGFβ3 even above the critical pH of 3.8 at which TGFβ3 aggregation is known to occur.

**Part E:**

The preparation of the citrate buffers pH 3.4 and 5.2 was according to methods standard in the art. The citrate buffer contained tri-sodium citrate, mannitol, HCl, 2% v/v benzyl alcohol and MQ water. The preparation of the
glycine buffer was according to methods standard in the art. The buffer contained 0.15% glycine, 4%w/w mannitol and hydrochloric acid pH 4.97.

This next part of the study was designed to examine the effects of a glycine-based buffer on TGFβ3 solubility at pH 5.0 in the absence of any gel. TGFβ3 was added to the appropriate buffer (see table below) at a final concentration of 20µg/mL. The samples were then centrifuged for 8 minutes at 1732Og and the supernatants and pellets analysed by rp-HPLC. The results are shown in Table 11.

Table 11:

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Recovery in the Supernatant</th>
<th>Total TGFβ3 recoverable (%) (mean, n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine pH4.97</td>
<td>85.7</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td>82.2</td>
<td>87.8</td>
</tr>
<tr>
<td>Sodium citrate/HCl pH5.2</td>
<td>0.15</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>53.3</td>
</tr>
<tr>
<td>Sodium citrate/HCl pH3.4</td>
<td>63.0</td>
<td>61.9</td>
</tr>
<tr>
<td></td>
<td>66.4</td>
<td>67.1</td>
</tr>
</tbody>
</table>

- Results are of duplicate samples

Omitting hypromellose from the formulated TGFβ3 had a minor impact on the solubility of TGFβ3, showing that the stabilising effect of glycine is not dependent on the presence of hypromellose.

**Part F:**

The aim of this part of the study was to correlate the recovery of TGFβ3 in the supernatant with A350 values recorded for TGFβ3 at pH 5.2 and 3.4 for both 20 and 125µg/mL samples.

The glycine gel was manufactured according to methods standard in the art, and pH was adjusted to 5.5 using NaOH. Nominal pH of the glycine gel was 3.5.

The gel contained the following components, 4% w/w mannitol, 0.15% w/w glycine, 0.017% v/w HCl, 1.9% w/w hypromellose and MQ water, pH 3.5. The gel was further pH adjusted to 5.5 using NaOH in this experiment; 2% v/v Benzyl alcohol was also added.
The glycine buffer contained 0.15% glycine, 4%w/w mannitol and hydrochloric acid to adjust pH to 3.4 and 5.2.

Glycine and mannitol were dissolved in water, pH was adjusted to 3.5 or 5.2 using HCl and the solution was then refrigerated. Hot water was added to hypromellose to form a slurry. The cold buffer was then added to the slurry with vigorous stirring to form the gel solvent.

Glycine gel pH 5.5 was diluted 1 in 4 with glycine buffer pH 3.4. Glycine gel pH 5.2 was diluted 1 in 4 with glycine buffer pH 5.5. All samples were diluted 1 in 4 and centrifuged at 5000G for 30 minutes. Supernatant was then removed and analysed by rp-HPLC. To measure aggregation, samples were unmodified and absorbance measured at 350nm. The results are shown in Table 12.

Table 12: Percentage recoveries in the supernatant in correlation with $A_{350}$ values recorded for TGFβ3 at pH 5.0 and 3.4 for both 20 and 125µg/ml samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Recovery in Supernatant Relative to that Recoverable (Mean, n=2)</th>
<th>Difference in $A_{350}$ (DP – placebo*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20µg/mL pH3.4</td>
<td>99.9</td>
<td>0.00104</td>
</tr>
<tr>
<td></td>
<td>101.5</td>
<td>0.00223</td>
</tr>
<tr>
<td>20µg/mL pH5.2</td>
<td>84.1</td>
<td>0.00947</td>
</tr>
<tr>
<td></td>
<td>87.4</td>
<td>0.00707</td>
</tr>
<tr>
<td>125µg/mL pH3.4</td>
<td>101.0</td>
<td>0.00246</td>
</tr>
<tr>
<td></td>
<td>100.8</td>
<td>0.00188</td>
</tr>
<tr>
<td>125µg/mL pH5.2</td>
<td>65.6</td>
<td>0.16707</td>
</tr>
<tr>
<td></td>
<td>53.8</td>
<td>0.25035</td>
</tr>
</tbody>
</table>

- *Placebo in this case is the formulation made in the absence of any TGFβ3.
- Results are of duplicate samples.
The recovery of soluble TGFβ3 at 20µg/mL, pH 5.2 was slightly lower than from the same formulation at pH 3.4. This indicates that the increased pH does still have an effect on TGFβ3 solubility, but that the solubility is dramatically improved by glycine. As the concentration of TGFβ3 was increased from 20 to 125µg/mL in glycine gel the impact of higher pH increased, which would be expected with a higher concentration of protein. Nonetheless, glycine does dramatically improve TGFβ3 solubility at pH values greater than the critical pH 3.8.

**Part G:**
The glycine gel and glycine buffer were prepared as described in Part F. The aim of this part of the study was to determine whether increasing the glycine concentration in gel from 20mM (0.15% w/v) up to 200mM (1.5% w/v) would improve the recovery of soluble TGFβ3 in the supernatant at the higher concentration of 125µg/ml at pH 5.2. Sample preparation and analysis was as described in Part F. The results are shown in Table 13.

Table 13: Proportion of TGFβ3 recovered in the supernatant.

<table>
<thead>
<tr>
<th>Glycine conc. in gel</th>
<th>% recovery supernatant (Mean, n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mM glycine (0.15% w/v)</td>
<td>84.6</td>
</tr>
<tr>
<td></td>
<td>84.4</td>
</tr>
<tr>
<td>40 mM glycine (0.3% w/v)</td>
<td>86.5</td>
</tr>
<tr>
<td></td>
<td>87.3</td>
</tr>
<tr>
<td>107mM glycine (0.8% w/v)</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td>88.3</td>
</tr>
<tr>
<td>200mM glycine (1.5% w/v)</td>
<td>81.7</td>
</tr>
<tr>
<td></td>
<td>81.4</td>
</tr>
</tbody>
</table>

- Results are of duplicate samples

Increasing the glycine concentration in the 125µg/ml TGFβ3 gel from 20mM to 200mM had no further effect on the solubility of TGFβ3. The insoluble TGFβ3 could be recovered in the pellet.

From this series of studies we confirmed that glycine in the gel formulation of TGFβ3 substantially prevented the aggregation of TGFβ3 at pH
values where TGFβ3 is normally insoluble. These results have implications for
the potential to formulate TGFβ3 in formulations above pH 3.8.

EXAMPLE 5. Pregnancy Outcome Study

When CBA/J mice are mated with DBA/2 mice, the progeny develop in a
placental environment which is quantitatively or qualitatively deficient in the
production of the anti-inflammatory Th2-type cytokines IL-4 and IL-10, while
there are increased levels of local inflammatory cytokines; these factors result
in a high incidence of spontaneous foetal resorption (miscarriage). The details
concerning the roles of these cytokines in the model were first published in
1990 (Chaouat G, Menu E, Clark DA, Dy M, Minkowski M, Wegmann TG.
Control of fetal survival in CBA x DBA/2 mice by lymphokine therapy. J Reprod

In this study recombinant TGFβ3 was administered vaginally (pv) in this
model of recurrent miscarriage to test the ability of TGFβ3 to reverse the high
levels of miscarriage by influencing the maternal immune status towards a pro-
pregnancy Th2-type response. A single dose of vehicle (10 microlitres of 0.1%
Bovine Serum Albumin in phosphate-buffered saline) or TGFβ3 (2 nanograms
in 10 microlitres of vehicle, 0.1% Bovine Serum Albumin in phosphate-buffered
saline at pH 7.4) was administered either before or after mating. Following
matting, the female mice were treated with an intra-peritoneal (ip) dose of
lipopolysaccharide (LPS) (1 microgram per 100 microlitres) to increase the
recurrent miscarriage rate. 13.5 days after mating the animals were sacrificed,
and the uteri were removed on day 13.5 of gestation for assessment of
(a) the number of embryos per mouse (the pregnancy rate), and
(b) the number of implantations and the number of resorptions (the
resorption rate).

In the vehicle-treated control group 15 animals were mated; of these 11
animals became pregnant, carrying a total of 97 implantations: 41% of these
implantations were subsequently lost. In the TGFβ3 treated group 14 animals
were mated; all animals became pregnant, producing 115 implantations, of
which 26% were subsequently lost. These results are summarized in Figure 1.
Human TGFβ3 significantly reduced the proportion of miscarriage in this CBA-
DBA/2 mouse model of recurrent miscarriage. The result is highly statistically
significant, as measured by the Chi-squared test (with Yates’ correction).
This result shows that a single dose of TGFβ3 administered to female mice lacking immune tolerance to paternal antigen, either before attempted conception or after attempted conception, actually prevents miscarriage and has no detrimental affect on pregnancy.

EXAMPLE 6. Additional formulations included within the scope of the invention

Formulation 1
TGFβ3 20µg/mL, mannitol (4% w/w), hypromellose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 4.8), purified water (to 100% w/w)

Formulation 2
TGFβ3 20µg/mL, mannitol (4% w/w), hypromellose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 4.0), purified water (to 100% w/w)

Formulation 3
TGFβ3 20µg/mL, mannitol (4% w/w), hypromellose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.0), purified water (to 100% w/w)

Formulation 4
TGFβ3 20µg/mL, glucose (4% w/w), hypromellose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 5
TGFβ3 20µg/mL, mannitol (4% w/w), methylcellulose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 6
TGFβ3 20µg/mL, mannitol (4% w/w), hydroxyethyl cellulose 4400 (1.8% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 7
TGFβ3 20µg/mL, mannitol (4% w/w), hydroxypropyl cellulose 4000 (1.9% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)
Formulation 8
TGFβ3 20µg/ml, mannitol (4% w/w), carmellose sodium, high viscosity grade (1.5% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 9
TGFβ3 20µg/mL, mannitol (4% w/w), tragacanth (2% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 10
TGFβ3 20µg/mL, mannitol (4% w/w), carbomer 940 (0.5% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 11
TGFβ3 20µg/mL, mannitol (4% w/w), poloxamer 407 (25% w/w), glycine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 12
TGFβ3 20µg/mL, mannitol (4% w/w), poloxamer 407 (25% w/w), arginine (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 13
TGFβ3 20µg/mL, mannitol (4% w/w), poloxamer 407 (25% w/w), CHAPS (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 14
TGFβ3 20µg/mL, mannitol (4% w/w), poloxamer 407 (25% w/w), PIPES (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w)

Formulation 15
TGFβ3 20µg/mL, mannitol (4% w/w), poloxamer 407 (25% w/w), lysergic acid (0.15% w/w), hydrochloric acid (to pH 5.5), purified water (to 100% w/w).

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.
CLAIMS:

1. A composition comprising a substantially purified TGFβ, a zwitterion, and a pharmaceutically acceptable carrier.

2. A composition according to claim 1, wherein the zwitterion is selected from the group consisting of an amino acid, a zwitterionic buffer compound, a zwitterionic detergent compound and an alkaloid.

3. A composition according to claim 2, wherein the amino acid is selected from the group consisting of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine and tyrosine.

4. A composition according to claim 2 or claim 3, wherein the amino acid is selected from the group consisting of arginine and glycine.

5. A composition according to any one of claims 2 to 4, wherein the amino acid is glycine.

6. A composition according to claim 2, wherein the zwitterionic buffer compound is selected from the group consisting of HEPES, PIPES, CAPS, or MOPS.

7. A composition according to claim 2, wherein the zwitterionic detergent compound is CHAPS.

8. A pharmaceutical composition according to claim 2, wherein the alkloid is a pharmacologically inert compound selected from the group consisting of a pyridine, a pyrrolidine, a tropane, a quinoline, an isoquinoline, a phenethylamine, an indole, an ergoline, a beta-carboline, a purine group or derivative thereof, a terpenoid, a steroid, a betaine, and a pyrazole.

9. A composition according to any one of claims 1 to 8, wherein the pH of the composition is above 2.0.
10. A composition according to any one of claims 1 to 9, wherein the pH of the composition is between 2.0 and 7.0.

11. A composition according to any one of claims 1 to 10, wherein the pH of the composition is between 3.7 and 7.0.

12. A composition according to any one of claims 1 to 11, wherein the pH of the composition is between 3.7 and 5.5.

13. A composition according to any one of claims 1 to 12, wherein the zwitterion is at a concentration of between 0.01% and 1% w/w.

14. A composition according to any one of claims 1 to 13, wherein the zwitterion is at a concentration of 0.15% w/w.

15. A composition according to any one of claims 3 to 5, wherein the glycine is at a concentration of between 0.01% and 1% w/w.

16. A composition according to any one of claims 3 to 5, wherein the glycine is at a concentration of between 0.15% and 1.5% w/w.

17. A composition according to any one of claims 3 to 5, wherein the glycine is at a concentration of 0.15% w/w.

18. A composition according to any one of claims 1 to 17, wherein the composition is in a liquid form.

19. A composition according to any one of claims 1 to 17, wherein the composition does not require reconstitution immediately prior to use.

20. A composition according to any one of claims 1 to 19, wherein the composition further comprises a pharmaceutically acceptable gel polymer.

21. A composition according to claim 20, wherein the zwitterion is formulated in the gel polymer.
22. A composition according to claim 20 or claim 21, wherein the gel polymer is selected from the group consisting of cellulose-based polymers, tragacanth polymers, xanthan gum polymers, acacia polymers, carbomer polymers, gelatin polymers, sodium alginate polymers, poloxomer polymers, polyethylene oxide polymers, polacrylamide polymers, and polyethylene glycol polymers.

23. A composition according to claim 22, wherein the gel polymer is a cellulose-based polymer.

24. A composition according to claim 23, wherein the cellulose-based polymer is selected from the group consisting of hypromellose, methylcellulose, hydroxypropylcellulose, ethylcellulose, hydroxyethylcellulose and carboxymethylcellulose.

25. A composition according to any one of claims 1 to 24, wherein the composition does not contain calcium chloride, calcium phosphate, potassium acetate, lithium acetate, ammonium acetate or ammonium bicarbonate.

26. A composition according to any one of claims 1 to 25, wherein the substantially purified TGFβ is a recombinant human TGFβ selected from the group consisting of TGFβ1, TGFβ2 and TGFβ3.

27. A composition according to any one of claims 1 to 26, wherein the substantially purified TGFβ maintains stability and/or solubility and/or bioactivity for at least 6 months when stored at between 2°C and 27°C.

28. A composition according to any one of claims 1 to 27, wherein the substantially purified TGFβ maintains stability and/or solubility and/or bioactivity for at least 6 months when stored at between 2°C and 8°C.

29. A composition according to any one of claims 1 to 28, wherein the concentration of the substantially purified TGFβ is between 0.01 µg/ml and 100 µg/ml.

30. An article of manufacture, comprising:
(1) a vial, cartridge, or vaginal applicator; and
(2) a composition according to any one of claims 1 to 28.

31. An article of manufacture according to claim 30, accompanied by, or labeled with, instructions that the article be used in the treatment of an infertility condition.

32. An article of manufacture according to claim 30 or claim 31, accompanied by, or labeled with, instructions that the article be stored at 27°C or below.

33. An article of manufacture, comprising:
   (1) a first vial or cartridge comprising a liquid composition comprising a buffering agent in an amount sufficient to maintain the pH of the composition above 3.7, and a zwitterion;
   (2) a second vial or cartridge comprising a dry powder composition comprising a freeze-dried TGFβ; and
   (3) instructions that the liquid composition is added to the dry powder composition prior to use.

34. A process for preparing a composition according to any one of claims 1 to 28, said process comprising the steps of adding a zwitterion and a pharmaceutically acceptable carrier to a substantially purified TGFβ.

35. A method of treating an infertility condition, said method comprising administering a composition according to any one of claims 1 to 28 to a prospective mother.

36. A method according to claim 35, said method comprising exposing a mucosal surface of said prospective mother to:
   (1) a composition according to any one of claims 1 to 28; and
   (2) semen or an MHC Class I antigen of a prospective father capable of eliciting a Th-1 response.

37. A method according to claim 35 or claim 36, wherein the infertility condition is implantation failure, recurrent miscarriage, pre-eclampsia or intrauterine growth retardation.
38. A method according to any one of claims 35 to 37, wherein the prospective mammalian mother lacks immune tolerance to a paternal antigen and exhibits a Th-1 immune profile indicating said lack of immune tolerance.

39. A method of reducing the adherence of TGFβ to the surface of a container, said method comprising the addition of a zwitterion to a composition comprising TGFβ.

40. A method of characterising TGFβ in a composition, said method comprising evaluating the TGFβ by means of an analytical method, wherein the composition comprising TGFβ also comprises a zwitterion.
A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
A61K 38/19 (2006.01)  A61K 31/198 (2006.01)  A61K 31/717(2006.01)  A61K 31/495 (2006.01)
A61K 47/22 (2006.01)  A61K 47/42 (2006.01)  A61P 15/08(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)

WPE ) S, MEDLINE & HCA: TGF-beta, zwITTERION, glycine, arginine, HEPES, PIPES, CAPS, stability, aggregate, adherence, precipitate and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See paragraph 28-3. 1. 85.</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>WO 2001/021766 A2 (CELL SCIENCE THERAPEUTICS) 29 March 2001</td>
<td>1-3, 6, 9</td>
</tr>
<tr>
<td></td>
<td>See page 23 L15-17 and page 26 L17-19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See pages 3 (L33-38), 6 and 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See pages 4 and 5</td>
<td></td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C

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
01 February 2008

Date of mailing of the international search report

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. +61 2 6285 7999

Authorized officer
Sean Harris
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No : (02) 6225 6164
INTERNATIONAL SEARCH REPORT

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1: Claims 1-38
A composition comprising TGF-beta and a zwitterion which is said to reduce aggregation and precipitation at a pH above 3.7.

Invention 2: Claim 39
A method for reducing the adherence of TGF-beta to the surface of a container with steps involving the addition of a zwitterion to a TGF-beta composition

Invention 3: Claim 40
A method for characterising TGF-beta in a composition comprising TGF-beta and a zwitterion

The only feature common to all of the claims is the TGF-beta/zwitterion composition. However this concept is not novel in the light of: US 2002/0064516

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention a posteriori.

1. [] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. X As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

Remark on Protest

[] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[] No protest accompanied the payment of additional search fees.
This Annex lists the known "A" publication level 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.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>US 2002064516</td>
<td>AU 35564/00 EP 1161257 US 6649168</td>
</tr>
<tr>
<td>WO 0121766</td>
<td>AU 20010424 EP 20020703 WO 20010329</td>
</tr>
<tr>
<td>WO 07113682</td>
<td></td>
</tr>
<tr>
<td>EP 1743649</td>
<td>AU 62846/98 CA 2283270 EP 1028743</td>
</tr>
<tr>
<td></td>
<td>US 7204978 US 2006177459 WO 9839021</td>
</tr>
</tbody>
</table>

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

END OF ANNEX