

CLAIMS

1. A composition comprising endometrial stem cells for use in a method for the treatment of diminished ovarian reserve, wherein said endometrial stem cells are
5 derived from an endometrial tissue sample.
2. The composition according to claim 1, wherein said endometrial stem cells are human autologous endometrial stem cells.
- 10 3. The composition according to claim 1 or 2, wherein said endometrial stem cells are endometrial mesenchymal stem cells
4. The composition according to claim 3, wherein said endometrial mesenchymal stem cells are positive for CD90, CD146 and CD105 markers and negative for the
15 CD34 and CD31 marker.
5. The composition according to claims 3 or 4, wherein said endometrial mesenchymal stem cells express Oct-4, CD146 and STRO-1.
- 20 6. The composition according to anyone of claims 1-5, wherein said composition is administered intra-ovarian or to at least to one ovary.
7. The composition according to anyone of claims 1-6, wherein said composition further comprising a physiologically relevant solution selected from the following
25 PBS solution, autologous serum, sterile normal saline or cell culture mediums at a pH of 7.2-7.4.
8. The composition according to anyone of claims 1-7, wherein said endometrial stem cells are at a concentration of 0.5-10 million cell number/mL, preferably 0.95-8
30 million cell number/mL, most preferably 1.5-6.5 million cell number/mL.
9. The composition according to anyone of claims 1-8, wherein said composition is administered in 1 to 5 doses, preferably 2 to 4 doses, at an amount of 0.5-2 million cell number per dose, preferably 0.8-1.3 million cell number per dose.
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10. A method for making a composition comprising endometrial, preferably endometrial mesenchymal stem cells, comprising the steps of:

- a. Submersing an endometrial tissue sample in a balanced salt solution;
 - b. Washing of the endometrial tissue sample, obtained from step a., with a buffered saline solution;
 - 5 c. Mincing the endometrial tissue sample, obtained from step b., to produce a minced endometrial tissue sample;
 - d. Digesting the minced endometrial tissue sample, obtained from step c., to produce a digestate, comprising epithelial cells and stem cells;
 - e. Centrifuging the digestate, obtained from step d;
 - 10 f. Separating through filtration or sorting the digestate, obtained from step e., into an epithelial cells fraction and an endometrial, preferably mesenchymal stem cells fraction;
 - g. Culturing the endometrial stem cells from step f. in a medium;
 - h. Characterisation of endometrial stem cells through flow cytometry, wherein said endometrial mesenchymal stem cells are positive for CD90, CD146 and CD105 markers and negative for CD34 and CD31 markers;
 - 15 i. Characterisation of endometrial stem cells through multipotency property, such as differentiation into osteocytes and adipocyte cells.
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11. The method for making a composition comprising endometrial mesenchymal stem cells, according to claim 10, wherein said endometrial mesenchymal stem cells are autologous endometrial mesenchymal stem cells.
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12. The method for making a composition comprising endometrial mesenchymal stem cells, according to claim 11, wherein in step f. the endometrial mesenchymal stem cells are cultured at 35-38 °C, 2-10% CO₂ and 95% humidity of air for 2 weeks, preferably at 37 °C, 5% CO₂ and 95% humidity of air for 2 weeks.
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13. The method for making a composition comprising endometrial mesenchymal stem cells, according to claim 11 or 12, wherein in step f. the medium is exchanged every 3 days.
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14. The method for making a composition comprising endometrial mesenchymal stem cells, according to anyone of claims 10-13, comprising the steps of:
 - a. Submersing the endometrial tissue sample in Hanks fluid;
 - b. Washing the endometrial tissue sample, obtained from step a., with PBS

- buffered phosphate saline comprising penicillin, amphotericin and streptomycin;
- 5 c. mincing the endometrial tissue sample, obtained from step b., to produce a minced endometrial tissue sample;
- d. Digesting the endometrial tissue sample, obtained from step b., with proteolytic collagenase to produce a digestate, comprising epithelial cells and stem cells;
- e. Centrifuging the digestate, obtained from step d.;
- 10 f. Separating through filtration the digestate into an epithelial cells fraction and a stem cells fraction using a 70 µm and 40 µm cell strainers; or sorting of stem cells by using specific mesenchymal stem cells markers such as CD146 and/or CD105 and/or CD90;
- g. Culturing the endometrial mesenchymal stem cells from step f. in an incubator at 37 °C, in a medium, 5 % CO₂ and 95 % humidity of air for 2 weeks;
- 15 h. Characterisation of endometrial mesenchymal stem cells through flow cytometry, wherein said endometrial mesenchymal stem cells are positive for CD90, CD146 and CD105 markers and negative for CD34 and CD31 markers;
- 20 i. Characterisation of endometrial mesenchymal stem cells through multipotency property, such as differentiation into osteocytes and adipocyte cells.
15. A method of treating diminished ovarian reserve, comprising the step of
- 25 administering a composition comprising endometrial, especially mesenchymal stem cells, according to anyone of claims 1-9.