

SUMMARY OF THE INVENTION

[0010] Methods, compositions and kits for determining the developmental potential of one or more embryos or pluripotent cells in one or more embryos or pluripotent cells are provided. These methods, compositions and kits find use in identifying embryos and oocytes in vitro that have a good developmental potential, i.e. the ability or capacity to develop into a blastocyst, which are thus useful in methods of treating infertility in humans, and the like.

[0010a] In a first aspect, there is provided a method for assessing whether one or more human embryos is likely to reach the blastocyst stage comprising: culturing one or more human embryos under conditions sufficient for embryo development; time lapse imaging said one or more embryos for the duration of at least one cytokinesis event or cell cycle; measuring at least one cellular parameter comprising: (a) the duration of the first cytokinesis; or (b) the time between the first and second mitosis; or (c) the time between the second and third mitosis; and identifying an embryo that is more likely to reach the blastocyst stage when said at least one cellular parameter for said embryo falls within: (i) a duration of the first cytokinesis that is about 0 to about 33 minutes; or (ii) a time between the first and second mitosis that is about 7.8 to 14.3 hours; or (iii) a time between the second and third mitosis that is about 0 to 5.8 hours, thereby assessing whether one or more human embryos is likely to reach the blastocyst stage.

[0011] In some ~~embodiment~~aspects of the ~~present disclosure~~invention, methods are provided for determining the developmental potential of an embryo or a pluripotent cell. In such ~~embodiment~~aspects, one or more cellular parameters of an embryo or pluripotent cell is measured to arrive at a cell parameter measurement. The cell parameter is then employed to provide a determination of the developmental potential of the embryo or pluripotent cell, which determination may be used to guide a clinical course of action. In some embodiments, the cell parameter is a morphological event that is measurable by time-lapse microscopy. In some embodiments, e.g. when an embryo is assayed, the one or more cell parameters is: the duration of a cytokinesis event, e.g. cytokinesis 1; the time interval between cytokinesis 1 and cytokinesis 2; and the time interval between cytokinesis 2 and cytokinesis 3. In certain embodiments, the duration of cell cycle 1 is also utilized as a cell parameter. In some embodiments, the cell parameter measurement is employed by comparing it to a comparable cell parameter measurement from a reference embryo, and using the result of this comparison to provide a determination of the developmental potential of the embryo. In some embodiments, the embryo is a human embryo. In some embodiments, the cell parameter is a gene expression level that is measured to arrive at a gene expression measurement. In some embodiments, the gene expression measurement is employed by comparing it to a gene expression measurement from a reference pluripotent cell or embryo or one or more cells therefrom, where result of this comparison is employed to provide a determination of the developmental potential of the pluripotent cell or embryo. In some embodiments, the embryo is a human embryo.

[0012] In some ~~embodiment~~ aspects of the ~~present disclosure~~ invention, methods are provided for ranking embryos or pluripotent cells for their developmental potential relative to the other embryos or pluripotent cells in the group. In such embodiments, one or more cellular parameters of the embryos or pluripotent cells in the group is measured to arrive at a cell parameter measurement for each of the embryos or pluripotent cells. The cell parameter measurements are then employed to determine the developmental potential of each of the embryos or pluripotent cells in the group relative to one another, which determination may be used to

guide a clinical course of action. In some embodiments, the cell parameter is a morphological event that is measurable by time-lapse microscopy. In some embodiments, e.g. when embryos are ranked, the one or more cell parameters are the duration of a cytokinesis event, e.g. cytokinesis 1; the time interval between cytokinesis 1 and cytokinesis 2; and the time interval between cytokinesis 2 and cytokinesis 3. In certain embodiments, the duration of cell cycle 1 is also measured. In some embodiments, the cell parameter is the expression level of one or more genes. In some embodiments, the one or more cell parameter measurements are employed by comparing the cell parameter measurements from each of the embryos or pluripotent cells in the group to one another to determine the developmental potential of the embryos or pluripotent cells relative to one another. In some embodiments, the one or more cell parameter measurements are employed by comparing each cell parameter measurement to a cell parameter measurement from a reference embryo or pluripotent cell to determine the developmental potential for each embryo or pluripotent cell, and comparing those developmental potentials to determine the developmental potential of the embryos or pluripotent cells relative to one another.

[0013] In some ~~embodiment~~aspects of the ~~present disclosure~~invention, methods are provided for providing embryos with good developmental potential for transfer to a female for assisted reproduction (IVF). In such ~~embodiment~~aspects, one or more embryos is cultured under conditions sufficient for embryo development. One or more cellular parameters is then measured in the one or more embryos to arrive at a cell parameter measurement. The cell parameter measurement is then employed to provide a determination of the developmental potential of the one or more embryos. The one or more embryos that demonstrate good developmental potential is then transferred into a female.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

[0015] **Figure 1** is a flow chart showing processes used to evaluate embryos.

[0016] **Figure 2** is a series of photographs showing cell cleavage and division over a period of 6 days. Images are labeled day 1 through day 6. Scale bar represents 50 μm .

CLAIMS

That which is claimed is:

1. A method for assessing good or poor developmental competence of a human embryo comprising

1) measuring cellular parameters of the human embryo in vitro, wherein said cellular parameters include:

- (a) the duration of the first cytokinesis;
- (b) the time interval between cytokinesis 1 and cytokinesis 2; or
- (c) the time interval between cytokinesis 2 and cytokinesis 3, and

2) determining that the human embryo has good developmental competence when,

- (a') the duration of the first cytokinesis is 0 to 30 minutes;
- (b') the time interval between the resolution of cytokinesis 1 and the onset of cytokinesis 2 is 8-15 hours; or
- (c') the time interval between the onset of cytokinesis 2 and the onset of cytokinesis 3 is 0-5 hours.

wherein said cellular parameters are measured by time-lapse microscopy.

2. The method of claim 1 wherein the cellular parameters include:

- (a) the duration of the first cytokinesis;
- (b) the time interval between cytokinesis 1 and cytokinesis 2; and
- (c) the time interval between cytokinesis 2 and cytokinesis 3.

3. The method of claim 1, wherein said cellular parameters further comprise the duration of cell cycle 1.

4. The method of claim 3, wherein good developmental competence of said human embryo is indicated when the duration of cell cycle 1 is 20 to 27 hours.

5. The method of claim 1, wherein poor developmental competence of said human embryo is indicated by

- (a) duration of the first cytokinesis is longer than 30 minutes;

- (b) the time interval between the resolution of cytokinesis 1 and the onset of cytokinesis is less than 8 hours or longer than 15 hours; and/or
- (c) the time interval between the onset of cytokinesis 2 and the onset of cytokinesis 3 is greater than 5 hours.

6. The method of claim 5, wherein said cellular parameters further comprise the duration of cell cycle 1.
7. The method of claim 6, wherein poor developmental competence of said human embryo is indicated when the duration of cell cycle 1 is longer than 27 hours.
8. The method of claim 1, wherein said embryos are produced by fertilization of oocytes in vitro.
9. The method of claim 8, wherein said oocytes are matured in vitro.
10. The method of claim 9, wherein said oocytes matured in vitro are supplemented with growth factors.
11. The method of claim 1, wherein the embryos have not been frozen prior to measuring the parameters.
12. The method of claim 1, wherein the embryos have been frozen prior to measuring the parameters.
13. The method of claim 1, wherein the human embryo has good developmental competence when the duration of the first cytokinesis is 0 to 30 minutes.
14. The method of claim 1, wherein the human embryo has good developmental competence when the time interval between the resolution of cytokinesis 1 and the onset of cytokinesis 2 is 8-15 hours.
15. The method of claim 1, wherein the human embryo has good developmental competence when the time interval between the onset of cytokinesis 2 and the onset of cytokinesis 3 is 0-5 hours.
16. The method of claim 1, wherein the human embryo has good developmental competence when the time interval between the resolution of cytokinesis 1 and the onset of cytokinesis 2 is 8-15 hours and the time interval between the onset of cytokinesis 2 and the onset of cytokinesis 3 is 0-5 hours.

CLAIMS:

1. A method for assessing whether one or more human embryos is likely to reach the blastocyst stage comprising:
 - culturing one or more human embryos under conditions sufficient for embryo development;
 - time lapse imaging said one or more embryos for the duration of at least one cytokinesis event or cell cycle;
 - measuring at least one cellular parameter comprising:
 - (a) the duration of the first cytokinesis; or
 - (b) the time between the first and second mitosis; or
 - (c) the time between the second and third mitosis; and identifying an embryo that is more likely to reach the blastocyst stage when said at least one cellular parameter for said embryo falls within:
 - (i) a duration of the first cytokinesis that is about 0 to about 33 minutes; or
 - (ii) a time between the first and second mitosis that is about 7.8 to 14.3 hours; or
 - (iii) a time between the second and third mitosis that is about 0 to 5.8 hours, thereby assessing whether one or more human embryos is likely to reach the blastocyst stage.
2. The method of claim 1 comprising: measuring:
 - (a) the duration of the first cytokinesis; and
 - (b) the time between the first and second mitosis; and identifying an embryo that is more likely to reach the blastocyst stage when:
 - (i) the duration of the first cytokinesis is about 0 to about 33 minutes; and
 - (ii) the time between the first and second mitosis is about 7.8 to 14.3 hours.
3. The method of claim 1 comprising: measuring:
 - (a) the duration of the first cytokinesis; and
 - (c) the time between the second and third mitosis, and identifying an embryo that is more likely to reach the blastocyst stage when:
 - (i) the duration of the first cytokinesis is about 0 to about 33 minutes; and
 - (iii) the time between the second and third mitosis is about 0 to 5.8 hours.

4. The method of claim 1 comprising: measuring:
 - (b) the time interval between the first and second mitosis; and
 - (c) the time between the second and third mitosis, and identifying an embryo that is more likely to reach the blastocyst stage when:
 - (ii) the time between the first and second mitosis is about 7.8 to 14.3 hours; and
 - (iii) the time between the second and third mitosis is about 0 to 5.8 hours.
5. The method of claim 1 comprising: measuring:
 - (a) the duration of the first cytokinesis; and
 - (b) the time between the first and second mitosis; and
 - (c) the time between the second and third mitosis, and identifying an embryo that is more likely to reach the blastocyst stage when:
 - (i) the duration of the first cytokinesis is about 0 to about 33 minutes; and
 - (ii) the time between the first and second mitosis is about 7.8 to 14.3 hours; and
 - (iii) the time between the second and third mitosis is about 0 to 5.8 hours.
6. The method of claim 1 comprising measuring the duration of the first cytokinesis and identifying that the duration of the first cytokinesis is about 0 to about 33 minutes.
7. The method of claim 1 comprising measuring the time between the first and second mitosis and identifying that the time between the first and second mitosis is about 7.8 to 14.3 hours.
8. The method of claim 1 comprising measuring the time between the second and third mitosis and identifying that the time between the second and third mitosis is about 0 to 5.8 hours.
9. The method of claim 1 wherein said at least one cellular parameter further comprises the duration of cell cycle 1.
10. The method of claim 9 wherein an embryo is more likely to reach the blastocyst stage when the duration of cell cycle 1 is about 20 to about 27 hours.
11. The method of claim 1 wherein said one or more embryos are produced by fertilization of oocytes in vitro.

12. The method of claim 11 wherein said oocytes are matured in vitro.
13. The method of claim 12 wherein said oocytes matured in vitro are supplemented with growth factors.
14. The method of claim 1 wherein said one or more embryos have not been frozen prior to measuring the at least one cellular parameter.
15. The method of claim 1 wherein said one or more embryos have been frozen prior to measuring the at least one cellular parameter.
16. The method of claim 1 wherein the measuring at least one cellular parameter is automated.
17. The method of claim 1 wherein the identifying that an embryo is more likely to reach the blastocyst stage is automated.
18. The method of claim 1 wherein said time lapse imaging acquires images that are digitally stored.
19. The method of claim 1 wherein said time lapse imaging employs darkfield illumination.
20. The method of claim 1 wherein said one or more human embryos are placed in a culture dish prior to culturing under conditions sufficient for embryo development.
21. The method of claim 20 wherein said culture dish comprises a plurality of microwells.
22. The method of claim 21 wherein said culture dish comprises from 1 to about 30 microwells.
23. The method of claim 21 wherein one or more human embryos is placed within a microwell prior to culturing under conditions sufficient for embryo development.

24. The method of claim 1 wherein the measuring is carried out at an imaging station.
25. The method of claim 1 wherein the one or more human embryos identified to be more likely to reach the blastocyst stage has the capacity to successfully implant into a uterus.
26. The method of claim 25 wherein the one or more human embryos with the capacity to successfully implant into the uterus has the capacity to go through gestation.
27. The method of claim 26 wherein the one or more human embryos with the capacity to go through gestation has the capacity to be born live.
28. The method of claim 1 comprising measuring the duration of the first cytokinesis and calculating that the duration of the first cytokinesis is about 0 to about 33 minutes.
29. The method of claim 1 comprising measuring the time between the first and second mitosis and calculating that the time between the first and second mitosis is about 7.8 to 14.3 hours.
30. The method of claim 1 comprising measuring the time between the second and third mitosis and calculating that the time between the second and third mitosis is about 0 to 5.8 hours.