could be from reservoirs of malignant cells in the body that were not killed with chemotherapy or radiation therapy or if the re-transplantation of cryopreserved gonadal tissue and cells harbored such malignant cells. As such, the optimal approach for such patients is to mature the cryopreserved gonadal tissue into functional sperm or eggs, and perform *in vitro* fertilization (with or without intracytoplasmic sperm injection or ICSI) with the natural or similarly *ex host* matured egg or sperm of the partner, and then after re-implant the fertilized embryo into female partner. This approach would be free from any possibility of tumor contamination as only the fertilized embryo formed from uncontaminated ex host matured sperm or egg.

[0010] Therefore, a germline cell banking system is needed in humans to restore reproductive potential in patients without the ability to bank mature sperm or ova and to mature the banked germline cells *ex host/ex vivo* or *ex host/in vivo* to produce mature sperm or ova.

SUMMARY OF THE INVENTION

[0011] The present disclosure provides methods to mature immature germline stem cells to produce mature reproductive cells.

[0012] In one embodiment, there is provided a method for maturing immature germline cells into haploid gametes *ex host* comprising the steps of:

obtaining immature germline cells from testes; and

culturing said immature germline cells *in vitro* in artificial semiferous tubules, wherein said culturing causes maturation of said immature germline cells into functional sperm.

[0012a] In one embodiment, there is provided a method for maturing immature germline cells into haploid gametes *ex host* comprising the steps of:

obtaining immature germline cells from ovaries; and

culturing said immature ovarian germline cells *in vitro* in the presence of granulosa cells and fibrin clot, wherein said culturing causes maturation of said immature ovarian germline cells into functional oocytes.

[0012b] In one embodiment disclosed herein, a method is provided for maturing immature germline cells into haploid gametes *ex host* comprising the steps of obtaining immature germline cells from testes or ovaries; and culturing the immature germline cells *in vitro* under conditions which mimic the conditions by which the cells mature *in vivo*, wherein the culturing causes maturation of the immature germline cells into functional sperm or oocytes.

[0012] In another embodiment, the immature germline cells are obtained from prepubertal patients. In another embodiment, the immature germline cells are cryopreserved prior to maturation. In another embodiment, the immature germline cells are germline stem cells or premeiotic germ cells.

[0013] In one embodiment, culturing comprises culture of immature testicular germline cells in artificial seminiferous tubules. In another embodiment, culturing comprises culture of immature testicular germline cells in the presence of at least one growth promoting factor selected from the group consisting of glial cell line-derived growth factor, fibroblast growth factor, leukemia inhibitor factor and epidermal growth factor. In another embodiment, culturing comprises culture of immature testicular germline cells in the presence of at least one maturation-inducing factor selected from the group consisting of follicle stimulating hormone, stem cell factor and retinoic acid.

We claim:

1. A method for maturing immature germline cells into haploid gametes *ex host* comprising the steps of:

obtaining immature germline cells from testes ~~or ovaries~~; and

culturing said immature germline cells *in vitro* ~~*or in vivo* in a surrogate animal outside of the original donor under conditions which mimic the conditions by which said cells mature *in vivo* in the original host,~~ in artificial seminiferous tubules, wherein said culturing causes maturation of said immature germline cells into functional sperm ~~or oocytes~~.

2. The method of claim 1 wherein said immature germline cells are obtained from prepubertal patients.

3. The method of claim 1 wherein said immature germline cells are cryopreserved prior to maturation.

4. The method of claim 1 wherein said immature germline cells are germline stem cells or premeiotic germ cells.

~~5. The method of claim 1 wherein said culturing comprises culture of immature testicular germline cells in artificial seminiferous tubules.~~

5~~6~~. The method of claim 1 wherein said culturing comprises culture of immature testicular germline cells in the presence of at least one growth promoting factor selected from the group consisting of glial cell line-derived growth factor, fibroblast growth factor, leukemia inhibitor factor, and epidermal growth factor.

6~~7~~. The method of claim 1 wherein said culturing comprises culture of immature testicular germline cells in the presence of at least one maturation-inducing factor selected from the group consisting of follicle stimulating hormone, stem cell factor, and retinoic acid.

7~~8~~. The method of claim 1 wherein said artificial seminiferous tubules comprise biocompatible tubing coated with extracellular matrix.

8~~9~~. The method of claim ~~8~~7 wherein said extracellular matrix is testicular extracellular matrix.

9~~10~~. The method of claim ~~9~~8 wherein said testicular extracellular matrix is autologous to said testicular germline cells.

~~11. The method of claim 1 wherein said culturing comprises culture of immature ovarian germline cells in the presence of granulosa cells and fibrin clot.~~

~~12. The method of claim 1 wherein said culturing comprises culture of immature ovarian germline cells cultured in the presence of growth promoting factors including glial~~

~~cell line-derived growth factor, fibroblast growth factor, leukemia inhibitor factor, epidermal growth factor and growth hormone.~~

~~12. The method of claim 1 wherein said culturing comprises culture of immature ovarian germline cells cultured in the presence of maturation inducing factors including follicle stimulating hormone, stem cell factor, and retinoic acid.~~

~~13. The method of claim 11 wherein said fibrin clot is autologous to said ovarian germline cells.~~

~~14. The method of claim 11 wherein said granulosa cells are autologous to said ovarian germline cells.~~

10. A method for maturing immature germline cells into haploid gametes *ex host* comprising the steps of:

obtaining immature germline cells from ovaries; and

culturing said immature ovarian germline cells *in vitro* in the presence of granulosa cells and fibrin clot, wherein said culturing causes maturation of said immature ovarian germline cells into functional oocytes.

11. The method of claim 10 wherein said immature germline cells are obtained from prepubertal patients.

12. The method of claim 10 wherein said immature germline cells are cryopreserved prior to maturation.

13. The method of claim 10 wherein said immature germline cells are germline stem cells or premeiotic germ cells.

14. The method of claim 10 wherein said culturing comprises culture of immature ovarian germline cells in the presence of at least one growth promoting factor selected from the group consisting of: glial cell line-derived growth factor, fibroblast growth factor, leukemia inhibitor factor, epidermal growth factor, and growth hormone.

15. The method of claim 10 wherein said culturing comprises culture of immature ovarian germline cells in the presence of at least one maturation inducing factor selected from the group consisting of: follicle stimulating hormone, stem cell factor, and retinoic acid.

16. The method of claim 10 wherein said fibrin clot is autologous to said ovarian germline cells.

17. The method of claim 10 wherein said granulosa cells are autologous to said ovarian germline cells.