

WHAT IS CLAIMED IS:

1. A method of generating a sterile sex-determined fish, comprising the steps of:
breeding (i) a fertile heterozygous mutated female fish having at least a first mutation and a second mutation with (ii) a fertile heterozygous mutated male fish having at least the first mutation and the second mutation; and
selecting a progeny that is homozygous by genotypic selection, the homozygous mutated progeny being the sterile sex-determined fish,
wherein the first mutation disrupts one or more genes that specify sexual differentiation and comprises: (a) a mutation in one or more genes that disrupt the synthesis of androgen and/or estrogen; and/or (b) a mutation in one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor, and
wherein the second mutation disrupts one or more genes that specify gamete function and comprises: (c) a mutation in one or more genes that disrupt spermiogenesis; and/or (d) a mutation in one or more genes that disrupt: (aa) oogenesis; (bb) folliculogenesis; or (cc) a combination thereof.
2. A method of generating a sterile sex-determined fish, comprising the step of:
breeding (i) a fertile homozygous mutated female fish having at least a first mutation and a second mutation with (ii) a fertile homozygous mutated male fish having at least the first mutation and the second mutation to produce the sterile sex-determined fish,
wherein the first mutation disrupts one or more genes that specify sexual differentiation and comprises: (a) a mutation in one or more genes that disrupt the synthesis of androgen and/or estrogen; and/or (b) a mutation in one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor,
wherein the second mutation disrupts one or more genes that specify gamete function and comprises: (c) a mutation in one or more genes that disrupt spermiogenesis; and/or (d) a mutation in one or more genes that disrupt: (aa) oogenesis; (bb) folliculogenesis; or (cc) a combination thereof, and
wherein the fertility of the fertile homozygous female fish and the fertile homozygous mutated male fish has been rescued.

3. The method of claim 2, wherein the fertility rescue comprises germline stem cell transplantation, and optionally sex steroid alteration, for example, an alteration of estrogen, or an alteration of an aromatase inhibitor.

4. The method of claim 3, wherein the germline stem cell transplantation comprises the steps of:

obtaining one or more germline stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more germline stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and transplanting the one or more germline stem cells into a germ cell-less recipient male fish, or into a germ cell-less recipient female fish,

or

obtaining one or more spermatogonial stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more oogonial stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and

transplanting the one or more spermatogonial stem cells or the one or more oogonial stem cells into an ovary of a germ cell-less fertile female fish or a testis of a germ cell-less fertile male fish.

5. The method of claim 4, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are homozygous for a null or loss of function mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

6. The method of claim 4, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.

7. The method of claim 4, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are homozygous for the mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

8. The method of claim 4, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.
9. The method of any one of claims 4-8, wherein the germ cell-less recipient male fish, the germ cell-less recipient female fish, the germ cell-less fertile female fish, and/or the germ cell-less fertile male fish, are created using at least one morpholino.
10. The method of claim 9, wherein the at least one morpholino is a morpholino that targets *dnd1*.
11. The method of any one of claims 1-10, wherein the sterile sex-determined fish is a sterile male fish.
12. The method of any one of claims 1-11, wherein the one or more genes that disrupt the synthesis of androgen and/or estrogen is: one or more genes that disrupt the expression of: aromatase Cyp19a1a, for example, one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof; Cyp17, for example, *cyp17l* or an ortholog thereof; or a combination thereof.
13. The method of any one of claims 1-12, wherein the one or more genes that disrupt spermiogenesis is: one or more genes that cause globozoospermia, for example, one or more genes that cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof, such as *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.
14. The method of any one of claims 1-10, wherein the sterile sex-determined fish is a sterile female fish.
15. The method of any one of claims 1-10 and 14, wherein the one or more genes that disrupt the expression of the aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

16. The method of any one of claims 1-10 and 14-15, wherein the one or more genes that disrupt:

(aa) oogenesis is one or more genes that disrupt the synthesis of estrogen, such as FSHR or an ortholog thereof; and/or

(bb) folliculogenesis is: one or more genes that disrupt the expression of vitellogenins, such as *vtgs* or an ortholog thereof; or a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

17. A method of generating a sterile sex-determined fish, comprising the step of:

breeding (i) a fertile female fish having one or more homozygous mutations with (ii) a fertile male fish having one or more homozygous mutations to produce the sterile sex-determined fish,

wherein the one or more mutations directly or indirectly disrupts spermiogenesis, and/or directly disrupts vitellogenesis, and

wherein the fertility of the fertile female fish and the fertile male fish have been rescued.

18. The method of claim 17, wherein the one or more mutations that directly or indirectly disrupts spermiogenesis is a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof.

19. The method of claim 17 or 18, wherein the one or more mutations that directly disrupts vitellogenesis is a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

20. The method of any one of claims 17-19, wherein the fertility rescue comprises germline stem cell transplantation, and optionally sex steroid alteration, for example, an alteration of estrogen, or an alteration of an aromatase inhibitor.

21. The method of claim 20, wherein the germline stem cell transplantation comprises the steps of:

obtaining one or more germline stem cells from a sterile homozygous male fish having at least the homozygous mutation or one or more germline stem cells from a sterile homozygous female fish having at least the homozygous mutation; and

transplanting the one or more germline stem cells into a germ cell-less recipient male fish, or into a germ cell-less recipient female fish.

22. The method of claim 21, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are homozygous for a null or loss of function mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

23. The method of claim 21, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.

24. The method of any one of claims 21-23, wherein the germ cell-less recipient male fish, and/or the germ cell-less recipient female fish are created using at least one morpholino.

25. The method of claim 24, wherein the at least one morpholino is a morpholino that targets *dnd1*.

26. The method of any one of claims 17-25, wherein the fertile female fish and the fertile male fish have an additional homozygous mutation that specifies sexual differentiation.

27. The method of claim 26, wherein the mutation that specifies sexual differentiation disrupts the expression of: (a) aromatase Cyp19a1a; (b) Cyp17, for example, a mutation in *cyp17l* or an ortholog thereof; (c) an inhibitor to aromatase Cyp19a1a, for example, a mutation in *Gsdf*, *dmrt1*, *Amh*, *Amhr*, or an ortholog thereof; or (d) a combination thereof.

28. The method of any one of claims 17-27, wherein the breeding step comprises hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.

29. A fertile homozygous mutated fish for producing a sterile sex-determined fish, the fertile homozygous mutated fish having at least a first mutation and a second mutation, wherein the first mutation disrupts one or more genes that specify sexual differentiation and comprises: (a) a

mutation in one or more genes that disrupt the synthesis of androgen and/or estrogen; and/or (b) a mutation in one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor, wherein the second mutation disrupts one or more genes that specify gamete function and comprises: (c) a mutation in one or more genes that disrupt spermiogenesis; and/or (d) a mutation in one or more genes that disrupt: (aa) oogenesis; (bb) folliculogenesis; or (cc) a combination thereof, and wherein the fertility of the fertile homozygous mutated fish has been rescued.

30. The fertile homozygous mutated fish of claim 29, wherein the fertility rescue comprises germline stem cell transplantation, and optionally sex steroid alteration, for example, an alteration of estrogen, or an alteration of an aromatase inhibitor.

31. The fertile homozygous mutated fish of claim 30, wherein the germline stem cell transplantation comprises the steps of:

obtaining one or more germline stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more germline stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and

transplanting the one or more germline stem cells into a germ cell-less recipient male fish, or into a germ cell-less recipient female fish,

or

obtaining one or more spermatogonial stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more oogonial stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and

transplanting the one or more spermatogonial stem cells in or the one or more oogonial stem cells into an ovary of a germ cell-less fertile female fish or a testis of a germ cell-less fertile male fish.

32. The fertile homozygous mutated fish of claim 31, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are homozygous for a null or loss of function mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

33. The fertile homozygous mutated fish of claim 31, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.
34. The fertile homozygous mutated fish of claim 31, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are homozygous for the mutation of the *dnf1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.
35. The fertile homozygous mutated fish of claim 31, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.
36. The fertile homozygous mutated fish of any one of claims 31-35, wherein the germ cell-less recipient male fish, the germ cell-less recipient female fish, the germ cell-less fertile female fish, and/or the germ cell-less fertile male fish, are created using at least one morpholino.
37. The fertile homozygous mutated fish of claim 36, wherein the at least one morpholino is a morpholino that targets *dnf1*.
38. The fertile homozygous mutated fish of any one of claims 29-37, wherein the sterile sex-determined fish is a sterile male fish.
39. The fertile homozygous mutated fish of any one of claims 29-38, wherein the one or more genes that disrupt the synthesis of androgen and/or estrogen is: one or more genes that disrupt the expression of: aromatase *Cyp19a1a*, for example, one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof; *Cyp17*, for example, *cyp17l* or an ortholog thereof; or a combination thereof.
40. The fertile homozygous mutated fish of any one of claims 29-39, wherein the one or more genes that disrupt spermiogenesis is: one or more genes that cause globozoospermia, for example, one or more genes that cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof, such as *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.

41. The fertile homozygous mutated fish of any one of claims 29-37, wherein the sterile sex-determined fish is a sterile female fish.
42. The fertile homozygous mutated fish of any one of claims 29-37 and 41, wherein the one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.
43. The fertile homozygous mutated fish of any one of claims 29-37 and 41-42, wherein the one or more genes that disrupt:
- (aa) oogenesis is one or more genes that disrupt the synthesis of estrogen, such as FSHR or an ortholog thereof; and/or
 - (bb) folliculogenesis is: one or more genes that disrupt the expression of vitellogenins, such as *vtgs* or an ortholog thereof; or a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.
44. A fertile fish having a homozygous mutation for producing a sterile sex-determined fish, wherein the mutation directly or indirectly disrupts spermiogenesis, and/or directly disrupts vitellogenesis, and wherein the fertility of the fertile fish has been rescued.
45. The fertile fish of claim 44, wherein the mutation that directly or indirectly disrupts spermiogenesis is a mutation in *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, or an ortholog thereof.
46. The fertile fish of claim 44 or 45, wherein the mutation that directly disrupts vitellogenesis is a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.
47. The fertile fish of any one of claims 44-46, wherein the fertile fish has a plurality of homozygous mutations that, in combination: directly or indirectly disrupt spermiogenesis; directly disrupt vitellogenesis; or both.

48. The fertile fish of any one of claims 44-47, wherein the fertility rescue comprises germline stem cell transplantation, and optionally sex steroid alteration, for example, an alteration of estrogen, or an alteration of an aromatase inhibitor.
49. The fertile fish of claim 48, wherein the germline stem cell transplantation comprises the steps of:
- obtaining one or more germline stem cells from a sterile homozygous male fish having at least the homozygous mutation or one or more germline stem cells from a sterile homozygous female fish having at least the homozygous mutation; and
 - transplanting the one or more germline stem cells into a germ cell-less recipient male fish, or into a germ cell-less recipient female fish.
50. The fertile fish of claim 49, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are homozygous for a null or loss of function mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.
51. The fertile fish of claim 49, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.
52. The fertile fish of any one of claims 49-51, wherein the germ cell-less recipient male fish, and/or the germ cell-less recipient female fish are created using at least one morpholino.
53. The fertile fish of claim 52, wherein the at least one morpholino is a morpholino that targets *dnd1*.
54. The fertile fish of any one of claims 44-53, wherein the fertile fish has an additional homozygous mutation that specifies sexual differentiation.
55. The fertile fish of claim 54, wherein the mutation that specifies sexual differentiation disrupts the expression of: (a) aromatase Cyp19a1a, for example, one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof; (b) Cyp17, for example, *Gsdf*, *dmrt1*, *Amh*, *Amhr*, or an ortholog thereof; (c) an inhibitor to aromatase Cyp19a1a, for

example, a mutation in *Gsdf*, *dmrt1*, *Amh*, *Amhr*, or an ortholog thereof; d) the synthesis of androgen and/or estrogen; or (e) a combination thereof.

56. The fertile fish of any one of claims 44-55, wherein producing a sterile sex-determined fish comprises a breeding step comprising hybridization or hormonal manipulation and breeding strategies, to specify sexual differentiation.

57. A method of making a fertile homozygous mutated fish that generates a sterile sex-determined fish, comprising the steps of: breeding (i) a fertile heterozygous mutated female fish having at least a first mutation and a second mutation with (ii) a fertile heterozygous mutated male fish having at least the first mutation and the second mutation;

selecting a progeny that is homozygous by genotypic selection; and

rescuing the fertility of the homozygous progeny,

wherein the first mutation disrupts one or more genes that specify sexual differentiation and comprises: (a) a mutation in one or more genes that disrupt the synthesis of androgen and/or estrogen; and/or (b) a mutation in one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor, and

wherein the second mutation disrupts one or more genes that specify gamete function and comprises: (c) a mutation in one or more genes that disrupt spermiogenesis; and/or (d) a mutation in one or more genes that disrupt: (aa) oogenesis; (bb) folliculogenesis; or (cc) a combination thereof.

58. The method of claim 57, wherein the fertility rescue comprises germline stem cell transplantation, and optionally sex steroid alteration, for example, an alteration of estrogen, or an alteration of an aromatase inhibitor.

59. The method of claim 58, wherein the germline stem cell transplantation comprises the steps of:

obtaining one or more germline stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more germline stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and

transplanting the one or more germline stem cells into a germ cell-less recipient male fish, or into a germ cell-less recipient female fish,

or

obtaining one or more spermatogonial stem cells from a sterile homozygous male fish having at least the first mutation and the second mutation or one or more oogonial stem cells from a sterile homozygous female fish having at least the first mutation and the second mutation; and

transplanting the one or more spermatogonial stem cells or the one or more oogonial stem cells into an ovary of a germ cell-less fertile female fish or a testis of a germ cell-less fertile male fish.

60. The method of claim 59, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are homozygous for a null or loss of function mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

61. The method of claim 59, wherein the germ cell-less recipient male fish and the germ cell-less recipient female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.

62. The method of claim 59, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are homozygous for the mutation of the *dnd1*, *Elavl2*, *vasa*, *nanos3*, or *piwi-like* gene.

63. The method of claim 59, wherein the germ cell-less fertile male fish and the germ cell-less fertile female fish are created using ploidy manipulation, hybridization, or exposure to high levels of sex hormones.

64. The method of any one of claims 59-63, wherein the germ cell-less recipient male fish, the germ cell-less recipient female fish, the germ cell-less fertile female fish, and/or the germ cell-less fertile male fish, are created using at least one morpholino.

65. The method of claim 64, wherein the at least one morpholino is a morpholino that targets *dnd1*.

66. The method of any one of claims 57-65, wherein the sterile sex-determined fish is a sterile male fish.

67. The method of any one of claims 57-66, wherein the one or more genes that disrupt the synthesis of androgen and/or estrogen is: one or more genes that disrupt the expression of: aromatase Cyp19a1a, for example, one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof; Cyp17, for example, *cyp17l* or an ortholog thereof; or a combination thereof.

68. The method of any one of claims 57-67, wherein the one or more genes that disrupt spermiogenesis is: one or more genes that cause globozoospermia, for example, one or more genes that cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof, such as *Gopc*, *Hiat1*, *Tjp1a*, *Smcp2*, *Csnk2a2*, and an ortholog thereof.

69. The method of any one of claims 57-65, wherein the sterile sex-determined fish is a sterile female fish.

70. The method of any one of claims 57-65 and 69, wherein the one or more genes that disrupt the expression of the aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.

71. The method of any one of claims 57-65 and 69-70, wherein the one or more genes that disrupt:

(aa) oogenesis is one or more genes that disrupt the synthesis of estrogen, such as FSHR or an ortholog thereof; and/or

(bb) folliculogenesis is: one or more genes that disrupt the expression of vitellogenins, such as *vtgs* or an ortholog thereof; or a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

72. The method of any one of claims 1-28 and 57-71 or the fertile fish of any one of claims 29-56, wherein the fish is a Tilapia fish.

73. A fish cell, comprising:
at least a first homozygous mutated gene and at least a second homozygous mutated gene, wherein:
the first mutation comprises: (a) a mutation in one or more genes that disrupt the synthesis of androgen and/or estrogen, and the second mutation comprises: (b) a mutation in one or more genes that disrupt spermiogenesis; or
the first mutation comprises: (c) a mutation in one or more genes that disrupt the expression of an aromatase Cyp19a1a inhibitor, and the second mutation comprises: (d) a mutation in one or more genes that disrupt: (aa) oogenesis; (bb) folliculogenesis; or (cc) a combination thereof.
74. The cell of claim 73, wherein the one or more genes that disrupt the synthesis of androgen and/or estrogen is: one or more genes that disrupt the expression of: aromatase Cyp19a1a, for example, one or more genes selected from the group consisting of *cyp19a1a*, *FoxL2*, and an ortholog thereof; Cyp17, for example, *cyp17l* or an ortholog thereof; or a combination thereof.
75. The cell of claim 73 or 74, wherein the one or more genes that disrupt spermiogenesis is: one or more genes that cause globozoospermia, for example, one or more genes that cause sperm with round-headed, round nucleus, disorganized midpiece, partially coiled tails, or a combination thereof, such as *Gopc*, *Hiat1*, *Tjp1a*, *Smap2*, *Csnk2a2*, and an ortholog thereof.
76. The cell of any one of claims 73-75, wherein the one or more genes that disrupt the expression of the aromatase Cyp19a1a inhibitor is one or more genes selected from the group consisting of *Gsdf*, *dmrt1*, *Amh*, *Amhr*, and an ortholog thereof.
77. The cell of any one of claims 73-76, wherein the one or more genes that disrupt:
(aa) oogenesis is one or more genes that disrupt the synthesis of estrogen, such as FSHR or an ortholog thereof; and/or
(bb) folliculogenesis is: one or more genes that disrupt the expression of vitellogenins, such as *vtgs* or an ortholog thereof; or a mutation in a gene encoding or regulating: Vitellogenin; Estrogen receptor1; Cytochrome p450, family 1, subfamily a; zona pellucida glycoprotein; Choriogenin H; Peroxisome proliferator-activated receptor; Steroidogenic acute regulatory protein, or an ortholog thereof.

78. The cell of any one of claims 73-77, wherein the fish cell is a Tilapia fish cell.
79. A method of producing the cell of any one of claims 73-78, comprising the steps of:
introducing at least the first homozygous mutation into a gene in the cell, and
introducing at least the second homozygous mutation into a gene in the cell.