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(54) Title: A CONTRACEPTIVE VACCINE BASED ON THE SPERM-ASSOCIATED PROTEIN CATSPER

(57) Abstract: A composition includes a contraceptive chimeric virus-like particle with an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain, with the antigenic carrier domain including human papillomavirus L1 capsid protein and the antigenic regions including one or more structural elements of the Catsper ion channel complex. When administered to a patient, the contraceptive vaccine stimulates production of anti-sperm antibodies that, upon binding to a sperm cell, inhibit the sperm cell's motility and thus inhibit the ability of the sperm cell to fertilize an egg cell. The induced immunoinfertility of the composition can be reversed for brief or extended lengths of time by overdosing the patient with a reversal agent lacking the antigenic carrier domain but having a protein sequence substantially identical to that of the one or more antigenic regions to sequester the anti-sperm antibodies.

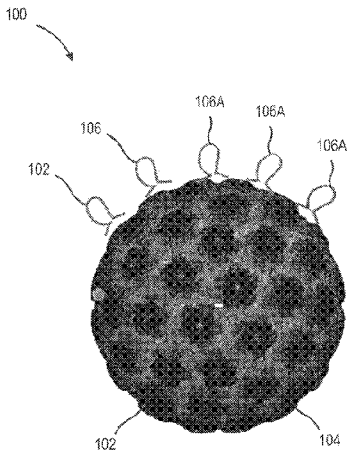

FIG. 1

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A CONTRACEPTIVE VACCINE BASED ON THE SPERM-ASSOCIATED PROTEIN CATSPER

CROSS REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/802,922, filed February 8, 2019, and 62/970,249, filed February 5, 2020, which are incorporated by reference as if disclosed herein in their entireties.

BACKGROUND

[0002] Almost half of all pregnancies worldwide are unwanted or mistimed. Current long-acting reversible contraceptive technology is focused on hormonal methods or surgery. Despite their overall effectiveness, there are myriad drawbacks to the contraceptive technologies available to women and men. By way of example, some women cannot tolerate hormonal contraception. Further, hormonal methods often require significant discipline or discomfort in the patient. For example, a regimen of pills or patches need to be taken or applied at regular intervals over the time that the contraceptive effect is desired. Implanted or intrauterine devices can cause mild to severe side-effects and complications, and still may require a clinical visit for reversal or for reimplantation.

[0003] What is desired, therefore, is an effective, long-lasting, and reversible contraceptive treatment with increased ease of use that also reduces the treatment-related burden on the patients desiring the contraceptive effects.

SUMMARY

[0004] Accordingly, some embodiments of the present disclosure relate to a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain. In some embodiments, the antigenic carrier domain includes one or more capsid proteins. In some embodiments, the one or more capsid proteins include L1 from human papillomavirus. In some embodiments, CGP amino acid residues are adjacent the N-terminal end of the one or more antigenic regions and GPC amino acid residues are adjacent the C-terminal end of the one or more antigenic regions. In some embodiments, the one or more antigenic

regions include structural elements of the Catsper ion channel complex. In some embodiments, the structural elements include at least a portion of one or more loops positioned between the transmembrane helical segments of the Catsper ion channel complex. In some embodiments, the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof. In some embodiments, the virus-like particle includes SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof.

[0005] Some embodiments of the present disclosure relate to a method of making a contraceptive chimeric virus-like particle including inserting a gene for an antigenic carrier protein into a plasmid, preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell, performing a polymerase chain reaction to amplify the chimeric gene, and synthesizing a virus-like particle from the chimeric gene. In some embodiments, the one or more antigenic regions include structural elements of the Catsper ion channel complex. In some embodiments, the structural elements include at least a portion of one or more loops positioned between the transmembrane helical segments of the Catsper ion channel complex. In some embodiments, the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof. In some embodiments, the virus-like particle includes SEQ. ID.

NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof.

[0006] Some embodiments of the present disclosure relate to a method for providing contraceptive treatment to a patient including preparing a composition including a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain, and administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions. In some embodiments, the antigenic carrier domain includes L1 from human papillomavirus. In some embodiments, the one or more antigenic regions include structural elements of the Catsper ion channel complex, wherein the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof. In some embodiments, the one or more antigenic regions includes SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof. In some embodiments, the composition is administered subcutaneously, intravenously, intranasally, or combinations thereof. In some embodiments, the method further includes administering a supplemental composition to the patient to reverse the effects of the composition, the supplemental composition including a reversal agent having a protein sequence substantially identical to that of the one or more antigenic regions. In some embodiments, the reversal agent includes one or more peptides, the one or more peptides include SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The drawings show embodiments of the disclosed subject matter for the purpose of illustrating the invention. However, it should be understood that the present application is not limited to the precise arrangements and instrumentalities shown in the drawings, wherein:

[0008] FIG. 1 is a schematic representation of a contraceptive chimeric virus-like particle according to some embodiments of the present disclosure;

[0009] FIG. 2 is a chart of a method of making a contraceptive chimeric virus-like particle according to some embodiments of the present disclosure;

[0010] FIG. 3A is a chart of a method of providing contraceptive treatment to a patient according to some embodiments of the present disclosure; and

[0011] FIG. 3B is a chart of a method of providing contraceptive treatment to a patient according to some embodiments of the present disclosure.

DETAILED DESCRIPTION

[0012] Referring now to FIG. 1, some embodiments of the present disclosure are directed to a vaccine for providing contraceptive treatment to an individual, user, patient, etc. In some embodiments, the contraceptive vaccine provides contraceptive benefits to the user with an effectiveness substantially equivalent to traditional hormonal contraceptives, implanted contraceptives, physical contraceptives, etc. In some embodiments, the contraceptive vaccine heightens an immune response of the user to a structure present on sperm cells. In some embodiments, the contraceptive vaccine heightens an immune response of the user to a structure of a protein or combination of proteins present on sperm cells. In some embodiments, the contraceptive vaccine stimulates production of anti-sperm antibodies in the user. In some embodiments, the contraceptive vaccine stimulates production of anti-sperm antibodies that, upon, binding to a sperm cell, inhibit the ability of the sperm cell to fertilize an egg cell. In some embodiments, the contraceptive vaccine stimulates production of anti-sperm antibodies that, upon, binding to a sperm cell, inhibit the sperm cell's motility. Without wishing to be bound by theory, when administered to women, the contraceptive vaccine stimulates production of anti-sperm antibodies, e.g., in the vaginal mucosa, which bind sperm and

prevent hyperactive motility in the sperm, rendering the sperm incapable of penetrating an egg. Again without wishing to be bound by theory, when administered to men, the contraceptive vaccine stimulates production of anti-sperm antibodies, e.g., in the epidydimis, which render the sperm substantially inert.

[0013] In some embodiments, the contraceptive vaccine includes a contraceptive chimeric virus-like particle (VLP) 100. In some embodiments, VLP 100 includes two or more domains 102. In some embodiments, VLP 100 includes an antigenic carrier domain 104. In some embodiments, antigenic carrier domain 104 includes one or more capsid proteins. In some embodiments, the one or more capsid proteins include L1 from human papillomavirus.

[0014] In some embodiments, VLP 100 includes an antigenic domain 106. In some embodiments, antigenic domain 106 includes one or more antigenic regions 106A. In some embodiments, antigenic regions 106A are positioned within the antigenic carrier domain 104. In some embodiments, antigenic regions 106A satisfy one or more of the following: are exposed on or about the sperm cell outer surface, are present only in sperm cells and in no other tissue in the human body, and are required for sperm function. In some embodiments, antigenic regions 106A include structural features or portions of structural features from a sperm cell. In some embodiments, antigenic regions 106A include structural elements of a protein or combination of proteins present on sperm cells. In some embodiments, antigenic regions 106A are present on sperm cell flagellum. In some embodiments, antigenic regions 106A include structural elements of the Catsper ion channel complex. In some embodiments, antigenic regions 106A include at least a portion of one or more loops positioned between the transmembrane helical segments of the Catsper ion channel complex. In some embodiments, antigenic regions 106A include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof. In some embodiments,

antigenic regions 106A include SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof.

[0015] In some embodiments, CGP amino acid residues are adjacent the N-terminal end of antigenic regions 106A. In some embodiments, GPC amino acid residues are adjacent the C-terminal end of one or more antigenic regions 106A. Without wishing to be bound by theory, these additional residues help stabilize antigenic regions 106A within antigenic carrier domain 104. In some embodiments, VLP 100 include SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof.

[0016] In some embodiments, VLP 100 is included in a composition. In some embodiments, the composition includes one or more preservatives, stabilizers, wetting agents, emulsifiers, buffers, fillers, etc. In some embodiments, the composition is configured for administration to a user subcutaneously, intravenously, intranasally, or combinations thereof.

[0017] Referring now to FIG. 2, some embodiments of the present disclosure are directed to a method 200 of making a contraceptive chimeric VLP. At 202, a gene for an antigenic carrier protein is inserted into a plasmid. At 204, overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell are prepared. As discussed above, in some embodiments, the one or more antigenic regions include structural elements of the Catsper ion channel complex, e.g., the structural elements include at least a portion of one or more loops positioned between the transmembrane helical segments of the complex. In some embodiments, the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations

thereof. At 206, a polymerase chain reaction is performed to amplify the chimeric gene. At 208, a VLP is synthesized from the chimeric gene. In some embodiments, the VLP is synthesized in bacterial or yeast cell culture. In some embodiments, the VLP includes SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof. In some embodiments, the VLP folds spontaneously. In some embodiments, the VLP is one or more of VLP 100 discussed above.

[0018] Referring now to FIG. 3A, some embodiments of the present disclosure are directed to a method 300 for providing contraceptive treatment to a patient. At 302, a composition including a contraceptive chimeric VLP including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain is prepared. In some embodiments, the chimeric VLP is one or more of VLP 100 discussed above. At 304, the composition is administered to a patient to heighten an immune response of the patient to the one or more antigenic regions. In some embodiments, the composition is administered to the patient subcutaneously, intravenously, intranasally, or combinations thereof. Without wishing to be bound by theory, contraceptive immunity is expected to last for about 1 to about 9 years, based on prior experience for women immunized against HPV.

[0019] Referring now to FIG. 3B, in some embodiments, at 306, a supplemental composition is administered to the patient to reverse the effects of the composition. In some embodiments, the supplemental composition includes a reversal agent having a protein sequence substantially identical to that of the one or more antigenic regions. In some embodiments, the supplemental composition does not include an antigenic carrier protein. In some embodiments, the reversal agent includes one or more peptides, the one or more peptides including SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof. In some embodiments, the supplemental composition includes one or more preservatives, stabilizers, wetting agents, emulsifiers, buffers, fillers, etc. Without wishing to be bound by theory, the induced immunoinfertility of the composition is reversed by sequestering the anti-sperm antibodies using overdosing with the supplemental composition. In some

embodiments, in the event that a sperm-immune individual wishes to recover fertility for a short period of time, a window of fertility may be created by the application of a reversal agent.

EXAMPLE

[0020] Design of chimeric L1. By way of example, chimeric L1 VLP forming proteins that include short antigenic segments from the sperm cationic ion channel Catsper were prepared. Antigenic segments of Catsper were predicted by identifying extracellular loops using the homology model. Site of insertion into the L1 gene were identified by multiple sequence alignment of homolog papilloma virus L1 sequences. Three candidate insertion sites were identified as sites of natural insertion/deletion. Bracketing CGP..GPC sequences were added to stabilize the inserted loop. DNA sequences were designed using DNAWorks.

[0021] Cloning of chimeric L1. The gene for HPV Type 11 L1 was synthesized using the assembly PCR method. The gene was inserted into the pET28a+ vector at NdeI and EcoRI cloning sites. The plasmid was grown and purified from cell culture using Escherichia coli strain DH5α. Chimeric constructs were made by amplifying the plasmid (inverse PCR) using overlapping oligos including the sequence of the desired antigenic region. Amplicons were transformed into DH5α cells for plasmid amplification, and were moved into BL21(DE3)pLysS cells for protein expression.

[0022] Isolation of inclusion body (IB). Recombinant proteins L1 protein of HPV Type 11 (L1), L1 with Catsper loop S3-S4 (P1), and L1 with Catsper-Epsilon loop 331-348 (C5) were expressed in E. coli. Recombinant proteins (L1, P1 and C5) were purified from inclusion bodies (IBs) isolated from E. coli Bl21(DE3) transformed by the plasmid constructs pET28a-L1-P1 and pET28a-L1-C5. Cells from a 500 ml culture of transformed E. coli cells, after induction for recombinant protein synthesis, were harvested. The wet cell biomass, approximately 1 g in weight, was suspended in 25 ml of buffer (50 mM Tris, 50 mM NaCl, pH 9.5) by gentle stirring. The suspension was subjected to sonication (15 cycles, 10 Sec on and 10 sec off in ice) and then centrifuged for 20 min at 12,000g at 4°C to pellet the IBs. Harvested IBs were washed twice with wash buffer 50 mM Tris (pH 9.5).

[0023] Solubilization and refolding. Comparatively purified IBs were solubilized in 5 ml of solubilization buffer (50 mM Tris (pH 9.5), 50 mM NaCl, 50 mM NaCl, 8 M urea), and stirred gently at room temperature for 2 h. The resultant suspension was centrifuged at 11000 rpm for 36 min at 25°C to collect total soluble protein. Solubilized IBs were refolded in 10 volumes of ice cold refolding buffer (50mM Tris, 50mM NaCl, 5% β-mercaptoethanol and 100mM L-Arginine (pH9.5)) using pulsatile dilution method at a rate of approximately 0.1ml/min. Refolded protein was centrifuged at 11000 rpm for 45 minutes at 4°C.

[0024] Dialysis and *in vitro* assembly of Virus-like particles (VLP). Refolded protein was filtered through 0.2µm filter (VWR) and dialyzed against dialysis buffer (50 mM Tris, 100mM NaCl, pH 9.5) using 12-14 kDa cut off dialysis membrane (Spectra/Por, VWR) at 4°C. NaCl concentration was increased stepwise up to 500 mM after three changes of dialysis buffer at 3 h intervals. Final dialysis was carried out for 2 h against high salt buffer (50 mM Tris, 500mM NaCl, pH 9.5).

[0025] Methods and system according to some embodiments of the present disclosure are directed to a contraceptive vaccine for providing long-lasting yet reversible contraceptive effects. The structure of the contraceptive vaccine stimulates an immune response to antigens present only in sperm cells, with the resulting anti-sperm antibodies effective to bind sperm and render those cells incapable of fertilization. The production and use of the contraceptive vaccine are simple. The need for hormone treatment and/or surgery are avoided as no action beyond vaccination is necessary to realize the benefits of the compositions of the present disclosure. Finally, the contraceptive immunity is easily and immediately reversible, with the immunity returning automatically to the contraceptive state with time as well.

[0026] Although the invention has been described and illustrated with respect to exemplary embodiments thereof, it should be understood by those skilled in the art that the foregoing and various other changes, omissions and additions may be made therein and thereto, without parting from the spirit and scope of the present invention.

CLAIMS

What is claimed is:

1. A contraceptive chimeric virus-like particle, comprising:

   an antigenic carrier domain; and

   one or more antigenic regions from a sperm cell in the antigenic carrier domain.

2. The chimeric virus-like particle according to claim 1, wherein the antigenic carrier domain includes one or more capsid proteins.

3. The chimeric virus-like particle according to claim 2, wherein the one or more capsid proteins include L1 from human papillomavirus.

4. The chimeric virus-like particle according to claim 1, wherein CGP amino acid residues are adjacent the N-terminal end of the one or more antigenic regions and GPC amino acid residues are adjacent the C-terminal end of the one or more antigenic regions.

5. The chimeric virus-like particle according to claim 1, wherein the one or more antigenic regions include structural elements of the Catsper ion channel complex.

6. The chimeric virus-like particle according to claim 5, wherein the structural elements include at least a portion of one or more loops positioned between the transmembrane helical segments of the Catsper ion channel complex.

7. The chimeric virus-like particle according to claim 6, wherein the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1

and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof.

8. The chimeric virus-like particle according to claim 7, wherein the virus-like particle includes SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof.

9. A method of making a contraceptive chimeric virus-like particle, comprising:

inserting a gene for an antigenic carrier protein into a plasmid;

preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell;

performing a polymerase chain reaction to amplify the chimeric gene; and

synthesizing a virus-like particle from the chimeric gene.

10. The method according to claim 9, wherein the one or more antigenic regions include structural elements of the Catsper ion channel complex.

11. The method according to claim 10, wherein the structural elements include at least a portion of one or more loops positioned between the transmembrane helical segments of the Catsper ion channel complex.

12. The method according to claim 11, wherein the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof.

13. The method according to claim 12, wherein the virus-like particle includes SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, or combinations thereof.

14. A method for providing contraceptive treatment to a patient, comprising:

preparing a composition including a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain; and

administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions.

15. The method according to claim 14, wherein the antigenic carrier domain includes L1 from human papillomavirus.

16. The method according to claim 14, wherein the one or more antigenic regions include structural elements of the Catsper ion channel complex, wherein the structural elements include at least a portion of: the loop between Catsper1 s1 and Catsper1 s2, the loop between Catsper2 s5 and Catsper2 p-loop, the loop between Catsper1 s3 and Catsper1 s4, the loop between Catsper2 s1 and Catsper2 s2, the loop between Catsper3 s1 and Catsper3 s2, the loop between Catsper1 s5 and Catsper1 p-loop, the loop between Catsper2 p-loop and Catsper2 s6, the loop between Catsper3 s3 and Catsper3 s4, the loop between Catsper3 s5 and Catsper3 p-loop, the loop between Catsper3 p-loop and Catsper3 s6, the loop between Catsper4 s1 and Catsper4 s2, the loop between Catsper4 p-loop and Catsper4 s6, Catsperδ loop 785-805, Catsperε loop 331-348, or combinations thereof.

17. The method according to claim 16, wherein the one or more antigenic regions includes SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof.

18. The method according to claim 14, wherein the composition is administered subcutaneously, intravenously, intranasally, or combinations thereof.

19. The method according to claim 14, further comprising:

administering a supplemental composition to the patient to reverse the effects of the composition, the supplemental composition including a reversal agent having a protein sequence substantially identical to that of the one or more antigenic regions.

20. The method according to claim 19, wherein the reversal agent includes one or more peptides, the one or more peptides include SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22, or combinations thereof.

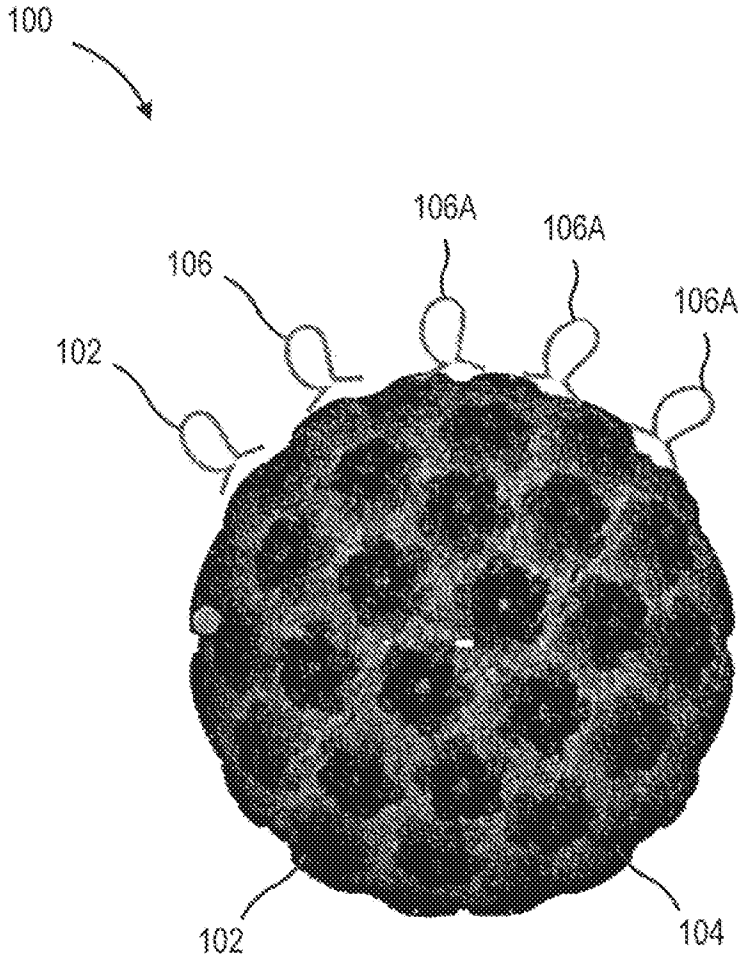

FIG. 1

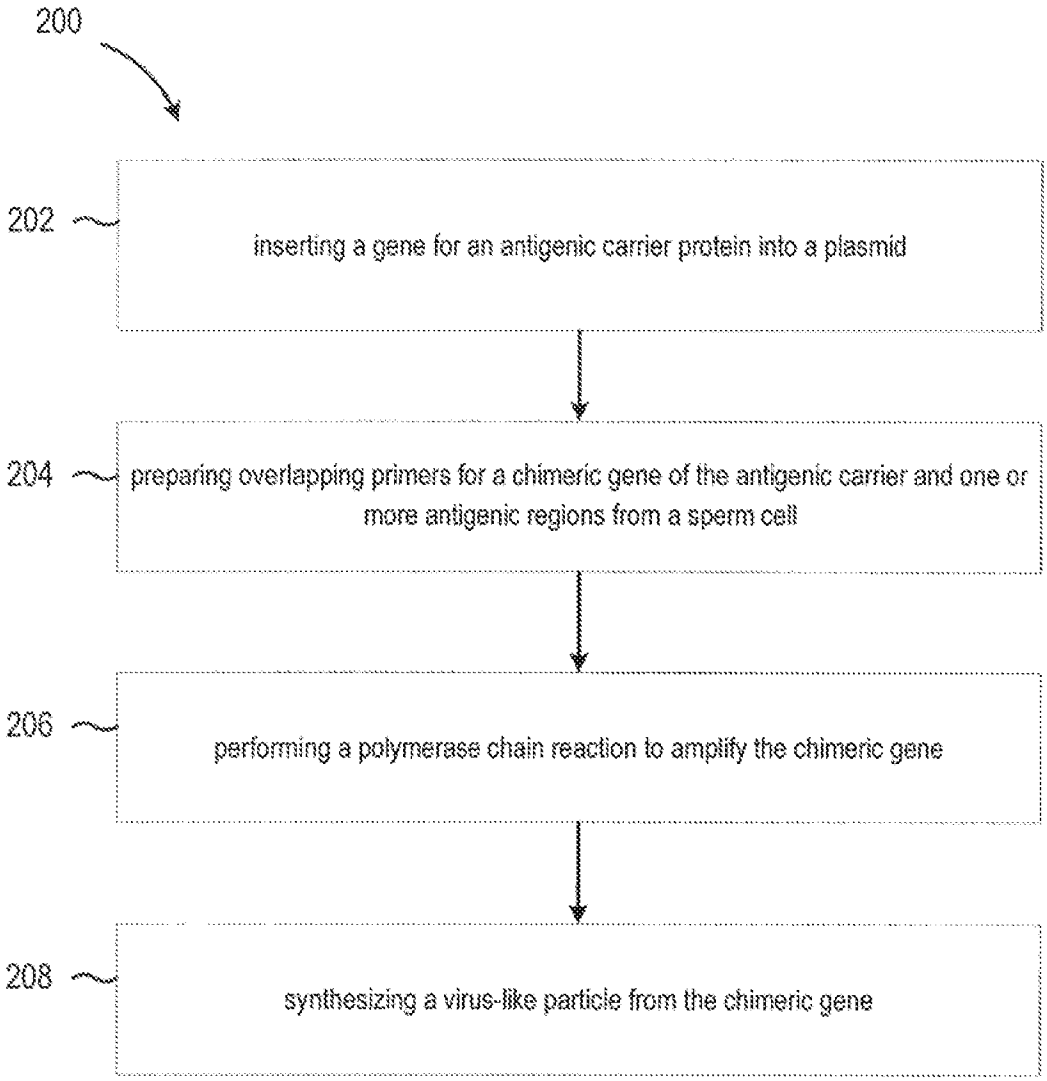
200
202
inserting a gene for an antigenic carrier protein into a plasmid
204
preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell
206
performing a polymerase chain reaction to amplify the chimeric gene
208
synthesizing a virus-like particle from the chimeric gene

FIG. 2A

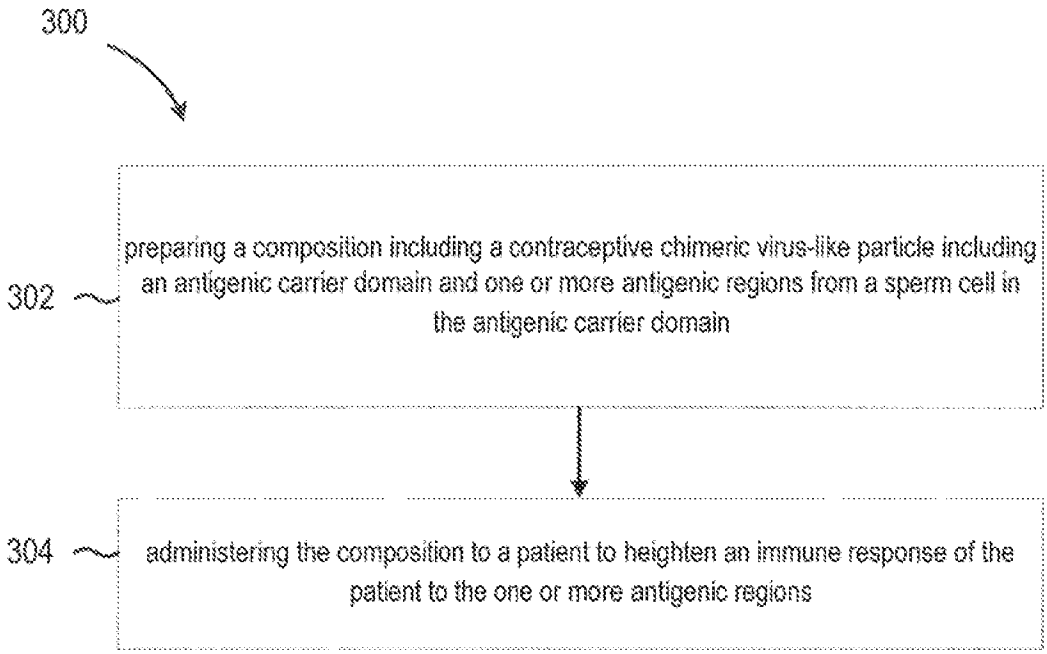
300
302
preparing a composition including a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain
304
administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions

FIG. 3A

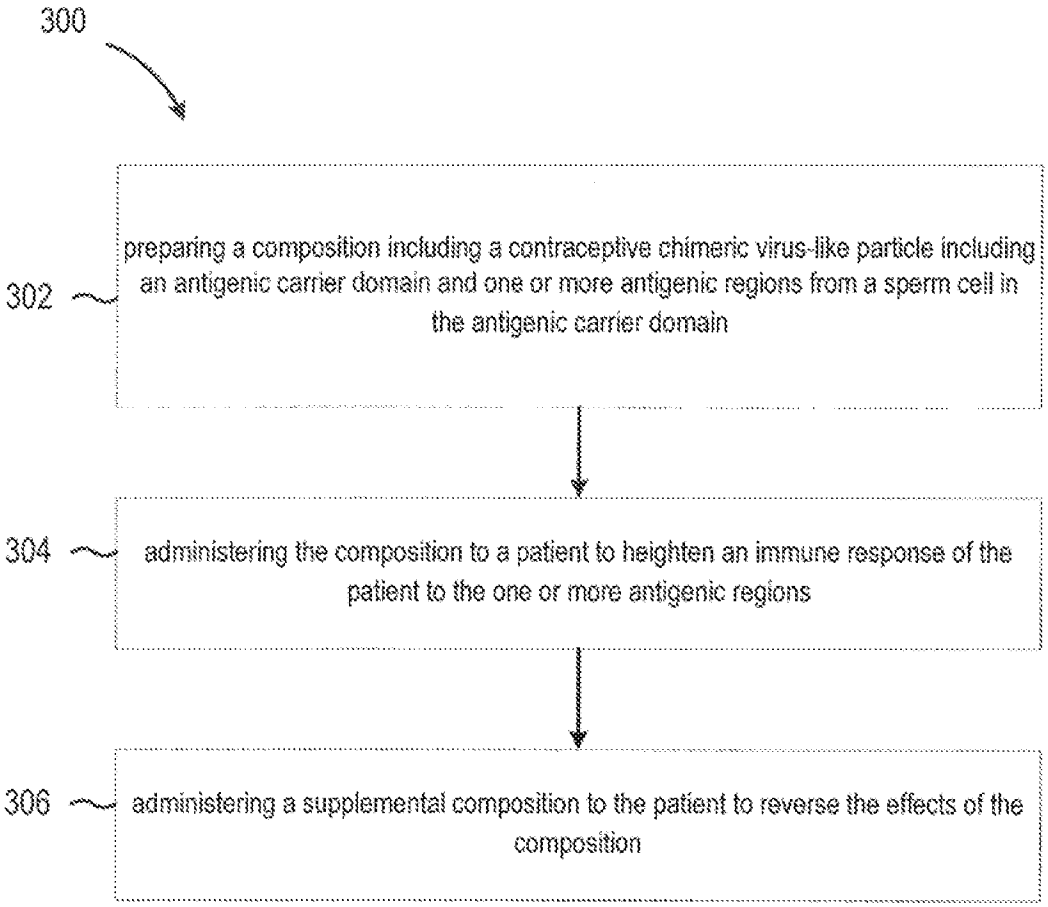
300
302
preparing a composition including a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain
304
administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions
306
administering a supplemental composition to the patient to reverse the effects of the composition

FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/17449

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/00; A61P 15/16, 15/18; A61K 35/52, 39/395; C07K 14/705; C12N 7/04 (2020.01)

CPC - A61K 39/0006; A61P 15/16, 15/18; A61K 35/52, 39/3955; C07K 14/705; C12N 5/061, 7/04

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	(BASORE, D et al.) Engineering Chimeric Virus-Like Particles for use as Vaccine Antigens. Conference abstracts (online). 2016 International Society for Vaccines Annual Congress. October 2016 [retrieved 22 April 2020]. Retrieved from the Internet: <URL: https://www.isv-online.org/images/docs/2016_book.pdf>; abstract P51	1-3, 14-15 ---------- 4-7, 9-12, 16-20 ---------- 8, 13
Y	US 2008/0014209 A1 (RICE, PA et al.) 17 January 2008; paragraph [0060]	4
Y	US 2017/0107269 A1 (CHILDREN'S MEDICAL CENTER CORPORATION) 20 April 2017; paragraphs [0015], [0021], [0028], [0063], [0101], [0111], [0124], [0158], [0167]; Fig. 1A	5-7, 10-12, 16-20
Y	WO 2008/025067 A1 (HEPGENICS PTY LTD) 6 March 2008; abstract; page 49, lines 4-10; page 50, lines 5-8	9-12
A	US 2018/0289792 A1 (MODERNATX. INC.) 11 October 2018; paragraphs [0030], [0091]	8, 13
A	(JELEN, MM) L1 protein [Human papillomavirus type 11]. Genbank entry (online). National Institute of Biotechnology Information.31 January 2016 [Retrieved on 6 May 2020]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/protein/CRF31054.1? >; page 1	8, 13
A	(MASHBURN, PB et al.) The role of antisperm antibodies in infertility. Fertility and Sterility. May 1994, Vol. 61, No. 5; pages 799-811; DOI: 10.1016/s0015-0282(16)56687-4	1-20
A	(YU, Q et al.) Construction of a Catsper1 DNA Vaccine and Its Antifertility Effect on Male Mice. PLoS One. 18 May 2015, Vol. 10, No. 5; pages 1-14; DOI: 10.1371/journal.pone.0127508	1-20

☐ 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

"D" document cited by the applicant in the international application

"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	Date of mailing of the international search report
13 May 2020 (13.05.2020)	17 JUL 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/17449

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+, Claims 1-20; loop between Catsper1 s1 and Catsper1 s2 (structural element); SEQ ID NO: 1 (virus-like particle sequence); SEQ ID NO: 8 (antigenic region) are directed toward a contraceptive chimeric virus-like particle with an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain, methods of making said VLP, and a composition for administering said VLP to a patient.

-***-Continued on supplemental page-***-

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

2. ☐ 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.:

1-20; loop between Catsper1 s1 and Catsper1 s2 (structural element); SEQ ID NO: 1 (virus-like particle sequence); SEQ ID NO: 8 (antigenic region)

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.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US20/17449

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The contraceptive chimeric virus-like particle will be searched to the extent that it encompasses a loop between Catsper1 s1 and Catsper1 s2 (first exemplary structural element), SEQ ID NO: 1 (first exemplary virus-like particle sequence), and SEQ ID NO: 8 (first exemplary antigenic region). Applicant is invited to elect additional structural element(s), virus-like particle sequence(s), and/or antigenic region(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), to be searched. Additional structural element(s), virus-like particle sequence(s), and/or antigenic region(s) will be searched upon the payment of additional fees. It is believed that claims 1-6, 7-8 (each in-part), 9-11, 12-13 (each in-part), 14-15, 16-17 (each in-part), 18-19, and 20 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a loop between Catsper1 s1 and Catsper1 s2 (structural element), SEQ ID NO: 1 (virus-like particle sequence), and SEQ ID NO: 8 (antigenic region). Applicants must specify the claims that encompass any additionally elected structural element(s), virus-like particle sequence(s), and/or antigenic region (s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a loop between Catsper2 s5 and Catsper2 p-loop (structural element).

No technical features are shared between the structural elements, virus-like particle sequence, and antigenic region sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a contraceptive chimeric virus-like particle, comprising: an antigenic carrier domain; and one or more antigenic regions from a sperm cell in the antigenic carrier domain; a method of making a contraceptive chimeric virus-like particle, comprising: inserting a gene for an antigenic carrier protein into a plasmid; preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell; performing a polymerase chain reaction to amplify the chimeric gene; and synthesizing a virus-like particle from the chimeric gene; a method for providing contraceptive treatment to a patient, comprising: preparing a composition including a contraceptive chimeric virus-like particle including an antigenic carrier domain and one or more antigenic regions from a sperm cell in the antigenic carrier domain; and administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions; these shared technical features are previously disclosed by the publication entitled 'Engineering Chimeric Virus-Like Particles for use as Vaccine Antigens' by Basore, et al. (hereinafter 'Basore') in view of WO 2008/025067 A1 (HEPGENICS PTY LTD) (hereinafter 'Hepgenics').

Basore discloses a contraceptive chimeric virus-like particle (CatSper (contraceptive) chimeric virus-like particle; abstract P51), comprising: an antigenic carrier domain (comprising major capsid protein L1 (antigenic carrier domain); abstract P51); and one or more antigenic regions from a sperm cell in the antigenic carrier domain (CatSper (one or more antigenic regions from a sperm cell in the antigenic carrier domain); abstract; P51); a method of making a contraceptive chimeric virus-like particle (producing a universal Dengue vaccine and an anti-sperm contraceptive vaccine; abstract P51), comprising: inserting a gene for an antigenic carrier protein (antigen is selected and designed into the β4β5 loop of L1 (comprising: inserting a gene for an antigenic carrier protein); abstract P51); a method for providing contraceptive treatment (producing an anti-sperm contraceptive vaccine (a method for providing contraceptive treatment); abstract P51), comprising: a contraceptive chimeric virus-like particle (CatSper (contraceptive) chimeric virus-like particle; abstract P51) including an antigenic carrier domain (comprising major capsid protein L1 (antigenic carrier domain); abstract P51) and one or more antigenic regions from a sperm cell in the antigenic carrier domain (CatSper (one or more antigenic regions from a sperm cell in the antigenic carrier domain) designed into the β4β5 loop of L1; abstract; P51).

Basore does not disclose a plasmid; preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell; performing a polymerase chain reaction to amplify the chimeric gene; and synthesizing a virus-like particle from the chimeric gene; a method for providing treatment to a patient, comprising: preparing a composition; and administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions.

Hepgenics discloses a plasmid (page 49, line 4); preparing overlapping primers for a chimeric gene (overlapping primers were each paired with an outside primer complementary to the plus strand of pCDL-w.t. or the minus strand of HCV construct in 2 first round PCR reactions using pfu enzyme; page 49, lines 4-10); performing a polymerase chain reaction to amplify the chimeric gene (page 49, lines 4-10); and synthesizing a virus-like particle from the chimeric gene (synthesizing a virus-like particle from the chimeric gene; page 50, lines 5-8); a method for providing a chimeric VLP a patient (administering an effective amount of the recombinant VLP to a subject; page 9, lines 1-9; page 20, lines 20-22), comprising: preparing a composition including a chimeric virus-like particle (preparing a vaccine that comprises the chimeric VLPs; page 9, lines 1-9; page 42, lines 27-30); and administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions (administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions; abstract; page 9, lines 1-9).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the disclosure of Basore, to include a plasmid; preparing overlapping primers for a chimeric gene of the antigenic carrier and one or more antigenic regions from a sperm cell; performing a polymerase chain reaction to amplify the chimeric gene; and synthesizing a virus-like particle from the chimeric gene; a method for providing treatment to a patient, comprising: preparing a composition; and administering the composition to a patient to heighten an immune response of the patient to the one or more antigenic regions, as disclosed by Hepgenics, in order to produce a superior VLP to generate an effective immune response against sperm cells providing an anti-sperm contraceptive for patients in need thereof.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Basore and Hepgenics references, unity of invention is lacking.