

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ABS GLOBAL, INC.,
Petitioner

v.

XY, LLC,
Patent Owner

Patent No. 7,208,265
Issued: April 24, 2007
Filed: January 5, 2000
Inventor: John L. Schenk

Title: METHOD OF CRYOPRESERVING SELECTED SPERM CELLS

Inter Partes Review No. IPR2017-02184

Declaration of Dr. Marvin M. Pace

U.S. Patent No. 7,208,265

I, Dr. Marvin M. Pace, do hereby declare and state, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: 10-10-2017

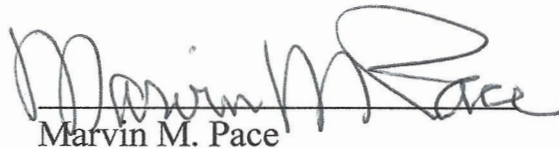

Marvin M. Pace

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Appendix A: List of Materials Considered

I. INTRODUCTION

A. Engagement

1. I have been retained by counsel for ABS Global, Inc. ("ABS"), as an expert witness in the above-captioned proceeding. I have been asked to render an opinion regarding the validity of claims 1-4, 8-20, 22, and 26-28 of U.S. Patent No. 7,208,265 ("the '265 patent"), which is submitted herewith as Exhibit 1001. The following is my written report on that topic.

B. Background And Qualifications

2. I have been actively involved in cryopreservation and artificial insemination of sperm and biological research related to reproduction in animals for the entirety of my adult life.

3. A complete list of my publications and a description of my other credentials and accomplishments are provided in my *curriculum vitae*, which is attached as Ex. 1004.

4. I began researching the cryopreservation of sperm in the 1960's and published my first paper in that field in 1967. *See* Ex. 1004, at 2. My research related to cryopreservation continued throughout my career. My Ph.D. thesis was entitled "A Study of Cryoprotective Properties of Egg Yolk in Semen." *See id*, at 1. This study tried to determine which beneficial components in egg yolk stabilized sperm membranes during the preservation process. I have published on

topics as diverse as how to protect sperm during the freezing process, how to thaw sperm after freezing, how the number of frozen sperm impact pregnancy rates, and how fertility rates of frozen sperm packaged in ampoules compare to frozen sperm packaged in straws.

5. My professional life has likewise been largely devoted to research concerning cryopreservation of sperm. I began work at American Breeders Service ("ABS") in 1971 and, other than a brief stint at Infigen, Inc., was employed by ABS (or a related company) until my retirement in 2007. Much of my work at ABS related to freezing sperm. For example, when I joined ABS, they had methods in place to freeze ampoules of sperm. However, the industry was moving from the use of ampoules as freezing containers to the use of plastic straws as freezing containers. I developed a straw freezing system that, to the best of my knowledge, is still being used by ABS today.

6. While at ABS, I was also involved in the development of extenders for semen that was to be frozen. For example, I developed extenders for horse semen that was to be frozen.

7. I also conducted experiments to refine extenders and processing procedures used in the preservation of sperm.

8. I also conducted experiments to try to understand the difference in fertility rates of frozen semen from different bulls or from the same bull over time.

9. While at ABS, I conducted over 300 laboratory experiments on semen physiology and cryopreservation of sperm.

10. I am the same Marvin M. Pace who prepared a declaration dated September 26, 2014, which was submitted in support of the Petitioner's challenge to the patentability of U.S. Patent No. 7,820,425 (the '425 patent) in IPR2014-01550. I understand that the '425 patent is related to the '265 patent at issue here and shares the same patent specification. My opinions substantially overlap with my opinions in IPR2014-01550, and I believe the challenged claims of the related '265 patent are unpatentable for the same or similar reasons.

C. Compensation And Prior Testimony

11. I am being compensated at a rate of \$150 per hour for my time spent on this matter. I am also being reimbursed for reasonable and customary expenses associated with my work in this proceeding. My compensation is not contingent on the outcome of this proceeding or the specifics of my testimony.

12. I have not previously testified as an expert witness, except for my deposition testimony in connection with IPR2014-01550.

D. Information Considered

13. My opinions are based on my over 45 years of education, research and experience in the field of cryopreservation and artificial insemination of sperm and biological research related to reproduction in animals, as well as my investigation and study of the relevant materials. In forming my opinions, I have considered the materials referred to herein and listed in Appendix A.

14. I may rely upon these materials and/or additional materials to rebut arguments raised by the patentee. Further, I may also consider adding additional documents and information in forming any necessary opinions – including documents I may not yet have reviewed and documents that have not yet been provided to me.

15. My analysis of the materials relating to this proceeding is ongoing and I will continue to review any new material as it is provided. This report represents only those opinions I have formed to date. I reserve the right to revise, supplement, and/or amend my opinions based on new information and on my continuing analysis of the materials referred to herein and listed in Appendix A.

II. LEGAL STANDARDS FOR PATENTABILITY

16. In expressing my opinions and considering the subject matter of the claims of the '265 patent, I am relying upon certain basic legal principles that counsel have explained to me.

17. Here, it is my understanding that the person of ordinary skill in the art is someone with at least a bachelor's degree in the biological sciences or a relevant field of engineering, and at least 3 years experience with methods of freezing sperm.

III. DESCRIPTION OF THE BACKGROUND TECHNOLOGY

A. The Artificial Insemination Industry

18. The agricultural industry relies heavily upon artificial insemination and has done so for many years. Artificial insemination permits the production of offspring which share the genetic attributes of parents of high quality. Artificial insemination is most often performed using frozen, unseparated sperm.

19. However, artificial insemination with either X chromosome bearing sperm or Y chromosome bearing sperm provides the industry with the option to increase the number of male or female progeny in a herd. For example, artificial insemination of a heifer (*i.e.*, a young female cow who has not previously given birth) with X chromosome bearing sperm allows dairy farmers to increase the number of female progeny. This is often desirable because female progeny are

generally more highly valued in the dairy industry, as only females produce milk. This technology can also be used to reduce the number of difficult births in heifers. Those of ordinary skill in the art understand that heifers pregnant with female calves have fewer difficulties giving birth because female calves are typically smaller than male calves. Thus, heifers are often inseminated with X chromosome bearing spermatozoa.

20. Current methods of artificial insemination in which only female or male progeny is desired rely upon two techniques. One technique involves the separation of X chromosome bearing sperm from Y chromosome bearing sperm, such as through the use of flow cytometry. The other technique involves the cryopreservation (*i.e.*, freezing) of sperm until it is needed to inseminate a female.

21. As recognized in the "Background of the Invention" section of the '265 patent, those of ordinary skill in the art knew how to both separate and freeze sperm long before the priority date of the '265 patent.

B. Methods Of Separating Sperm Were Well Known

22. The '265 patent states that a variety of techniques to separate sperm into X chromosome bearing or Y chromosome bearing populations were known to those of skill in the art before the alleged invention of the '265 patent:

The invention is exemplified herein with selection based on sex-type, and sex-selected sperm for use in the invention can be obtained using any selection strategy

that takes advantage of slight differences in characteristics between X- and Y-type sperm. Exemplary sex-selection methods include magnetic techniques (see, e.g., U.S. Pat. No. 4,276,139), columnar techniques (see, e.g., U.S. Pat. No. 5,514,537) gravimetric techniques (see, e.g., U.S. Pat. No. 3,894,529, reissue Pat. No. 32350, U.S. Pat. Nos. 4,092,229, 4,067,965, and 4,155,831). Sex-selection based on differences in electrical properties is disclosed in U.S. Pat. No. 4,083,957, and techniques that select based on differences in electrical and gravimetric properties are discussed in U.S. Pat. Nos. 4,225,405, 4,698,142, and 4,749,458. U.S. Pat. Nos. 4,009,260 and 4,339,434 describe selection based on differences in motility. Biochemical techniques relying on antibodies are disclosed in U.S. Pat. Nos. 4,511,661, 4,999,283, 3,687,803, 4,191,749, 4,448,767, whereas U.S. Pat. Nos. 5,021,244, 5,346,990, 5,439,362, and 5,660,997 describe selection based on differences in membrane proteins.

Ex. 1001 ('265 patent), 4:20-39.

23. The '265 patent states that the previously known method of flow cytometry was a preferred method for separating sperm into X chromosome bearing or Y chromosome bearing populations.

Flow cytometry is a preferred method for separating cells from mixed populations based on differential staining

with fluorescent dyes or binding to fluorescently labeled molecules, such as antibodies or nucleic acids. In fluorescence activated cell sorting ("FACS"), cells are "sorted" into different populations based on the fluorescence intensity upon irradiation. FACS can be used for sex-selection of sperm because the X chromosome contains slightly more DNA than the Y chromosome. When sperm are stained with a fluorescent DNA-binding dye, X-chromosome bearing sperm absorb more dye than Y chromosome bearing sperm and the two populations can therefore be separated by FACS. This strategy was discussed in U.S. Pat. No. 4,362,246 and significantly expanded upon in U.S. Pat. No. 5,135,759 (issued to Johnson). Separation has been enhanced through the use of high-speed flow cytometers, such as the MoFlo® flow cytometer produced by Cytomation, Inc. (Ft. Collins, Colo.) and described in U.S. Pat. Nos. 5,150,313, 5,602,039, 5,602,349, and 5,643,796, as well as in PCT Publication No. WO 96/12171.

Ex. 1001 ('265 patent), 4:40-59.

24. The '265 patent recognized that flow cytometry had been used years before the '265 patent priority date to sort sperm. *See above*, ¶ 48, U.S. Patent No. 5,135,759 issued to Lawrence A. Johnson ("Johnson 1992"), Ex. 1008.

Johnson 1991 describes a method of flow cytometry that was already known in the industry. *Compare id.*, at cover, *with* Ex. 1001 ('265 patent), at cover.

25. As explained in Johnson 1992, flow cytometry allows for the separation of X chromosome bearing sperm cells from Y chromosome bearing sperm cells based on "relative DNA content." Ex. 1008 (Johnson 1992), 2:64-67. Specifically, "[t]he X chromosome is larger and contains slightly more DNA than does the Y chromosome." *Id.*, 1:60-62. This difference in chromosome size means that an X chromosome bearing sperm cell contains more DNA than a Y chromosome bearing sperm cell. In bulls, for example, the X bearing chromosome is 3.8% bigger than Y chromosome bearing sperm. *Id.*, 1:62-64.

26. As Johnson also explains, flow cytometry uses this difference in DNA quantity in the separation process and shows separation of both sperm nuclei, *id.*, 3:38-41, and intact sperm, *id.*, 4:5-18, 7:35-41. In either case, the sperm cells are stained with a fluorochrome that is nontoxic to sperm, such as Hoechst bisbenzimidazole H 33342. *Id.*, 4:27-31. Then, the sperm cells are passed through a laser beam and the flow cytometer measures and analyzes the amount of fluorescent light given off by the cells as they pass through the laser. *Id.*, 3:4-6. "Because the X chromosome contains more DNA than the Y chromosome, the female sperm (X) takes up more dye and gives off more fluorescent light than the male sperm (Y)." *Id.*, 3:10-13. The flow cytometer can then electrostatically

direct X chromosome bearing sperm into one collection container and Y chromosome bearing sperm into another container. *Id.*, 3:46-49.

27. Well before 1991, it was also known in the art to remove seminal plasma prior to sorting using the Johnson technique. For example, U.S. Patent No. 5,021,244 ("Spaulding"), Ex. 1031, provides an Example 1 that describes "Sorting Sperm by Flow Cytometry" using the Hoechst 33342 fluorescent dye to distinguish between the X-sperm and Y-sperm. Ex. 1031 (Spaulding), 7:43-10:8. Example 1 describes the "[p]reparation of [s]perm" prior to sorting. *Id.*, 9:15-36. As part of the preparation, the sperm was washed three times and then centrifuged to remove the seminal plasma. *Id.*, 9:25-27 ("We centrifuged the cells at 483Xg for 20 minutes and resuspended them to remove seminal plasma proteins."). This preparation process would have removed all, or nearly all, of the seminal plasma prior to sorting. After removal of the seminal plasma and addition of the dye, the sperm was then sorted using a flow cytometer. *Id.*, 9:38-10:8. After sorting, two viable subpopulations enriched for X- or Y-sperm, respectively, were obtained. *Id.*, 9:65-66.

28. Another paper that shows removing seminal plasma prior to sorting with flow cytometry is, E.F. Fugger, S.H. Black, K. Keyvanfar and J.D. Schulman, "Births of normal daughters after MicroSort sperm separation and intrauterine insemination, in-vitro fertilization, or intracytoplasmic sperm

injection," Human Reprod., Vol. 13, No. 9 (1998) ("Fugger"), Ex. 1007. Fugger notes that "[f]resh or frozen semen specimens were provided for sorting."

Ex. 1007 (Fugger), at 2367. As part of that process, the sperm to be sorted were "extended, centrifuged, resuspended, [and] filtered" Ex. 1007 (Fugger) at 2367. Fugger refers to another paper, for the specific details of these preparative steps, along with the centrifugation conditions. *See* Ex. 1032 (Johann 1989) cited by Fugger at 2367. The Johann paper cited by Fugger shows that the samples of sperm were subjected to centrifugation conditions that would have resulted in removal of all, or nearly all, of the seminal plasma. Ex. 1032 (Johann 1989) at 686 (describing three sequential centrifugations at 750 x g for 15 minutes, 200 x g for 5 minutes and 200 x g for 5 minutes – each followed by removal of the supernatant and resuspension).

29. Prior to 1999, centrifugation was also used after the sorting step to remove sheath fluid. *See, e.g.*, Ex. 1005 (U.S. Patent No. 7,195,920 ("Seidel 1997")), 13:61-64.

C. Methods Of Freezing Sperm Were Well Known

30. Sperm has been frozen, and thereafter used to inseminate female animals, since at least the 1950's. Indeed, the '265 patent recognized that "techniques for freezing unselected sperm are well known." Ex. 1001 ('265 patent), 2:6-7.

31. Polge and Rowson 1952, for example, reported on their experiments freezing bovine sperm in 1952. Ex. 1009 (Polge, C. and Rowson, L.E.A., "Long-term Storage of Bull Semen Frozen at Very Low Temperatures (-79° C.)," in "Report of The II. International Congress of Physiology and Pathology of Animal Reproduction and of Artificial Insemination," Vol. 3 (1952) ("Polge and Rowson 1952")). They reported that their work enabled them to "offer strong support to the idea that if living cells can be cooled to temperatures low enough to arrest biochemical activity and subsequently rewarmed without damage it should be possible to preserve the cells at these low temperatures almost indefinitely." *Id.* at 90-91.

32. The Polge and Rowson work was premised on the fundamental discovery that "glycerol and some related compounds have the property of protecting spermatozoa of several species when frozen at -79° C." Ex. 1009 (Polge and Rowson 1952) at 91. Indeed, in my opinion, the discovery that glycerol had cryoprotective properties revolutionized the artificial insemination industry.

33. Polge and Rowson reported two sets of experiments. In the first, they extended bovine sperm 1:1 with citrate-yolk buffer (3.92% sodium citrate, pH 6.7 plus 50% egg) and 30% glycerol, resulting in a final glycerol concentration of 15%. Ex. 1009 (Polge and Rowson 1952) at 92. The extender and sperm were maintained at approximately 30° C. *Id.* The sperm was thereafter

slowly frozen by placing ampoules of semen and extender inside a small vacuum flask containing alcohol and immersing that flask containing the semen in a larger freezing bath at -79° C. *Id.* The next day, the sperm inside the ampoules was thawed and used to inseminate cows. *Id.* Surprisingly to Polge and Rowson, this frozen sperm resulted in a 0% pregnancy rate. *Id.* at 92-93.

34. Polge and Rowson thereafter devoted their attention to "improving the conditions of freezing." Ex. 1009 (Polge and Rowson 1952) at 93. What they discovered was that cooling the sperm and allowing the sperm to equilibrate with the extender prior to freezing increased the viability of the sperm. *Id.* Specifically, the sperm was extended with yolk-citrate buffer and allowed to cool to 5° C for 4-6 hours. *Id.* It was then further extended with a volume of citrate buffer containing 20% glycerol, resulting in a final concentration of 10% glycerol. *Id.* The mixture was allowed to stand overnight at 5° C. *Id.* Sperm was then transferred to ampoules in one ml portions, and the ampoules were cooled slowly over 45 minutes to -79° C. *Id.* at 94. Sperm inside the ampoules was thawed before use and then used to artificially inseminate cows, resulting in a 79% pregnancy rate. *Id.*

35. Polge and Rowson concluded "it has been conclusively shown that the freezing of bull spermatozoa at -79° C. under certain conditions is

compatible with the retention of fertilizing capacity." Ex. 1009 (Polge and Rowson 1952) at 94.

36. Polge and Rowson also reported that slow cooling was preferable to fast cooling. Specifically, they reported that bull or goat sperm "frozen fairly rapidly to -79° C. . . . showed only a negligible revival of spermatozoa on thawing." Ex. 1009 (Polge and Rowson 1952) at 91. On the other hand, if "the glycerol-treated semen was cooled slowly to -79° C the sample could be revived to show a considerable degree of motility." *Id.* The best revival rates were achieved when the semen was frozen over the course of about 45 minutes (*i.e.*, about -1.12 degrees per minute), but other freezing rates were also found via experimentation to be acceptable. *Id.* at 91-93.

37. After the discovery of Polge and Rowson, frozen semen quickly became the norm. Indeed, "[p]retreatment of bovine semen [in an extender] with glycerol, slow cooling, freezing and storage in liquid nitrogen" was used "extensively and routinely, with great success." Ex. 1010 (LaSalle, B., "Introduction," The Integrity of Frozen Spermatozoa, Procs. of a Round-Table Conf. held on Apr. 6-7, 1976, Nat'l Acad. of Scis. (1978) ("LaSalle 1978")) at 3. This "made possible great progress in livestock improvement by increasing several thousandfold the potential progeny of better-selected sires or donors and by allowing ample time for extensive evaluation and testing for genetic qualities,

freedom of genetic defects and agents of infectious diseases, for international transport, and for long-range breeding programs." *Id.*

1. Extenders used in freezing were known

38. The concentration of sperm in a normal ejaculate of semen is typically high and contains more sperm than are needed to inseminate a female. Thus, those of skill in the art typically add an "extender" to sperm. An extender has two major functions: (1) it preserves the fertility of the sperm, and (2) it increases the total volume so that the proper dose of sperm cells can be conveniently packaged and used. Because extending the volume of sperm results in a reduced concentration of sperm, some individuals in the art used the term "diluent" instead of the term "extender." Most people in the industry, however, used the term "extender" to convey the idea that the dilution process did not reduce overall pregnancy rates. Similarly, some individuals in the art used the term "extend" to refer to the process of adding an extender.

39. An extensive body of work regarding extenders had been developed before the priority date of the '265 patent. It was, for example, well known among those of ordinary skill in the art that an ideal extender for semen should "aid in the preservation and longevity" of the sperm. Ex. 1011 (H.A. Herman and F.W. Madden, "The Artificial Insemination of Dairy and Beef Cattle:

A Handbook and Laboratory Manual," 6th Ed. (1980) ("Herman and Madden 1980")) at 85.

40. Additionally, there was generally a consensus that an ideal extender should, among other things: (a) maintain proper osmotic pressure; (b) contain constituents that prevent deleterious shifts in pH; (c) contain constituents that provide protection against the harmful effects of cooling and temperature shock; (d) contain antibiotics that inhibit bacterial growth; and (e) provide energy and nutrients. Ex. 1006 (Salisbury *et al.*, Physiology of Reproduction and Artificial Insemination in Cattle, 2nd Ed. (1978) ("Salisbury")) at 498-99; Ex. 1011 (Herman and Madden 1980) at 85; Ex. 1012 (Graham, E.F., "Fundamentals of the Preservation of Spermatozoa," The Integrity of Frozen Spermatozoa, Procs. of a Round-Table Conf. held on Apr. 6-7, 1976, Nat'l Acad. of Scis. (1978) ("Graham 1978")) at 9, 15.

41. A variety of ingredients were available and known by those of ordinary skill in the art to perform each of the functions of an ideal extender.

42. For example, various buffers were known to help maintain osmolality and pH and were routinely used by those of skill in the art in extenders. Examples of buffers used extensively well before the priority date of the '265 patent include MES, PIPES, TES, HEPES, BES, MOPS, TRICINE, sodium

citrate and TRIS. Ex. 1006 (Salisbury) at 499; Ex. 1011 (Herman and Madden 1980) at 86; Ex. 1012 (Graham 1978) at 10.

43. It was also well known long before the priority date of the '265 patent that certain ingredients could be used in extenders to protect against the harmful effects of cooling and freezing, including glycerol, egg yolk and milk. Glycerol was widely reported to be a cryoprotectant and the key discovery that allowed mammalian sperm to be frozen. Ex. 1006 (Salisbury) at 500, 511-14; Ex. 1011 (Herman and Madden 1980) at 86; Ex. 1012 (Graham 1978) at 15; Ex. 1013 (Berndtson, W.E. and Pickett, B.W, "Techniques for The Cryopreservation and Field Handling of Bovine Spermatozoa," in The Integrity of Frozen Spermatozoa, Procs. of a Round-Table Conf. held on Apr.6-7, 1976, Nat'l Acad. of Scis. (1978) ("Berndtson and Pickett 1978")) at 62. Specifically, it was believed the glycerol helps to control ice crystal formation within the sperm cells.

44. It was also known that the "optimal concentration of glycerol depends upon several factors, including the type of extender and freezing rate." Ex. 1013 (Berndtson and Pickett 1978) at 62. It had been reported that "levels of approximately 7 percent glycerol are optimal for citrate-yolk and tris-yolk extenders, and levels of 11 to 13 percent for fresh and reconstituted skim-milk extenders." *Id.*; *see also* Ex. 1006 (Salisbury) at 500. Similarly, it had been reported that glycerol levels of 6% and 8% "resulted in maximum sperm survival

during freezing." Ex. 1014 (Bulletin 621, "Preservation of Bull Semen at Sub-Zero Temperatures," U. of Ill. Ag. Exp. Station (1957) ("Bulletin 621")) at 18; *see also* Ex. 1011 (Herman and Madden 1980) at 102.

45. Milk and egg yolk were also reported to help sperm resist cold shock. Ex. 1006 (Salisbury) at 499-500; Ex. 1011 (Herman and Madden 1980) at 86-87; Ex. 1012 (Graham 1978) at 10-11, 14. Cold shock occurs when sperm are subjected to sudden cooling and results in damage to the cell membrane. The tail of a sperm cell that has been exposed to cold shock often wraps itself around the body of the sperm cell. Milk and egg yolk were believed to help prevent damage to the cell membrane.

46. It was also well understood that one or more antibiotics were useful ingredients in extenders. Penicillin, sulfanilamide, streptomycin, neomycin, and linco-spectin were all commonly used antibiotics long before the priority date of the '265 patent. Ex. 1006 (Salisbury) at 469-72; Ex. 1011 (Herman and Madden) at 86, 94; Ex. 1012 (Graham 1978) at 11.

47. It was also well understood that antibiotics were mandatory. Indeed, members of the National Association of Animal Breeders were required to include antibiotics in semen used for artificial insemination. Certified Semen Services ("CSS"), a subsidiary of the National Association of Animal Breeders, would periodically make recommendations as to the type of antibiotics in semen.

For example, at the Proceedings of the 11th Technical Conference on Artificial Insemination and Reproduction, held on April 25-26, 1986, CSS announced that in order to comply with CSS Minimum Requirements, organizations were required to include a combination of tylosin, gentamycin and linco-spectin in sperm samples used for artificial insemination. Ex. 1015 (Doak, "CSS Implementation of New Antibiotic Combination," Procs. of the 11th Tech. Conf. on Artificial Insemination and Reprod. (1986) ("Doak 1986")) at 42-43. Prior to that announcement, the CSS standards required organizations to use penicillin, dihydrostreptomycin and polymyxin B sulfate. *See id.* at 42. Likewise, individuals of skill in the art would have understood that semen could not be shipped to other states unless the semen samples were treated with antibiotics.

48. Energy sources suitable for use in extenders that were well known long before the priority date of the '265 patent included simple sugars such as glucose and fructose. Ex. 1006 (Salisbury) at 501-02; Ex. 1011 (Herman and Madden 1980) at 86.

49. Individuals of skill in the art understood that there was no one "best" extender. Ex. 1011 (Herman and Madden 1980) at 85. Rather, individuals had their own preferred extenders, based on experience, species from which the sperm was obtained, and other factors, such as ease of use and cost. Ex. 1006 (Salisbury) at 447. By the time of the priority date of the '265 patent, persons of

ordinary skill in the art, using routine experimentation, could readily determine whether an extender worked satisfactorily in any given situation.

2. Dilution ratios were known

50. Not only was much known about the materials needed to preserve sperm by freezing, much was also known about the methods that would result in viable sperm after freezing. Ex. 1011 (Herman and Madden 1980) at 102-104; Ex. 1012 (Graham 1978) at 19-22.

51. For example, those of skill in the art knew that the "dilution ratio for freezing varies with species, freezing container and technique, thawing and insemination techniques, and volume and sperm concentration needed for fertility." Ex. 1012 (Graham 1978) at 13. A person of ordinary skill in the art would have been well-equipped to determine, for any particular technique, how many sperm cells should be frozen, and it was known that both "[h]igh and low concentrations of sperm cells may be frozen successfully, depending on technique and species." *Id.*

3. The need for slow cooling prior to freezing was known

52. Long before 1999, the benefits of allowing sperm to slowly cool prior to freezing were well-known. Bovine, equine and porcine semen, among others, were routinely slow cooled to prevent cold shock. Ex. 1006 (Salisbury) at 509-511; Ex. 1012 (Graham 1978) at 12; *see also* Ex. 1011 (Herman

and Madden 1980) at 102 (instructing that sperm should be slowly cooled for 3-5 hours); Ex. 1014 (Bulletin 621) at 19-20 ("One can see that sperm survived freezing better when the diluted semen was cooled to 4.5° C. before the glycerol was added. . . . Thus, it appears that cooling to refrigerator temperature (4.5° C.) before adding the glycerol should be a part of the routine procedure."). This is because it was known that "[r]apid decreases in semen temperature from 37° C to 5° C cause cold shock" Ex. 1012 (Graham 1978) at 14. Thus, it was known that the "optimum cooling rate is one that prevents cold shock but is fast enough to maintain healthy viable sperm cells." *Id.*; *see also* Ex. 1013 (Berndtson and Pickett 1978) at 61 ("Cold shock can be prevented by slow cooling in the presence of protective agents."); Ex. 1006 (Salisbury) at 509 (noting that sperm should be "cooled slowly for maximum liveability of the cells.").

4. The need to equilibrate the sperm prior to freezing was known

53. By the 1970's numerous investigators had determined that the fertility of frozen sperm was enhanced when the semen was equilibrated with glycerol for several hours prior to freezing. *See, e.g.*, Ex. 1006 (Salisbury) at 514 (noting that fertility trials "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period of several hours."). The optimal length of equilibration varied, but generally fell between 2 and 18 hours.

Id. at 514-15 (recommending 4-18 hours of storage at 5° C before freezing based on then-existing knowledge); *see also* Ex. 1011 (Herman and Madden 1980) at 102 (instructing that sperm be equilibrated after being extended with a glycerol-containing extender). It had also been reported that "[a]t 4.5° C., little variation in motility following freezing and thawing was found after equilibration times of 2, 6 and 18 hours." Ex. 1014 (Bulletin 621) at 20.

5. It was known that sperm should not be frozen too quickly

54. Polge and Rowson, in their seminal work with glycerol, showed that bull or goat semen frozen rapidly to -79° C showed only a negligible revival on thawing. Ex. 1009 (Polge and Rowson 1952) at 91. They demonstrated that sperm "cooled slowly" to -79° C showed a "considerable degree of motility" upon thawing. *Id.* They reported that an 80% survival rate was achieved when bull semen was frozen from 0° C to -79° C over a span of about 45 minutes and that "[v]ariations in the rate of freezing seemed to have little effect, although optimal revivals occurred if the rate of cooling between 5° C. and -15° C. did not exceed 2° per minute." *Id.* at 93; Ex. 1006 (Salisbury) at 519-23 (discussing freezing rates). *See also, supra*, at ¶ 60.

55. Other investigators successfully used different rates of freezing. *See, e.g.*, Ex. 1011 (Herman and Madden 1980) at 102 (reporting a rate of 1° C per minute from 0° C to -15° C and then 3° to 4° per minute to -70° C).

D. It Was Well Known That Viable Sperm Could Be Obtained After Separation and Freezing

56. Well before 1999, it was known that sperm that had undergone processing designed to separate the sperm could thereafter be frozen using known techniques and that the resulting sperm would be viable.

57. For example, Fugger *et al.* reported in 1998 that they had successfully separated X chromosome bearing sperm obtained from humans from Y chromosome bearing sperm by flow cytometry, frozen it, and thereafter successfully used it to achieve pregnancy in women. Ex. 1007 (Fugger); *see also, infra*, at ¶¶ 207-208, 210-215.

58. Likewise, Seidel *et al.* published an abstract in January 1999 reporting that X chromosome bearing sperm obtained from bulls, separated by flow cytometry from Y chromosome bearing sperm, and frozen "by standard procedures" were viable and resulted in a 51% pregnancy rate. Ex. 1016 (Seidel *et al.*, "Insemination of Heifers with Sexed Frozen or Sexed Liquid Semen," 51:1 Theriogenology: An Int'l J. of Animal Reprod., 400 (1999) ("Seidel 1999")). Based on proceeding papers distributed after the proceedings, I believe that Seidel also announced at the Proceedings of the 17th Technical Conference on Artificial Insemination and Reproduction, held in September 1998 in Madison, Wisconsin, that in recent experiments he had "found that pregnancy rates after artificial insemination of heifers with sexed, frozen-thawed semen were similar to

pregnancy rates using sexed, unfrozen semen." Ex. 1017 (Seidel, "Fertility of Bulls on the Edge of the Dose-Response Curve for Numbers of Sperm Per Inseminate," in Procs. of the 17th Technical Conference on Artificial Insemination and Reprod. (1998) ("Seidel 1998")) at 23.

59. U.S. Patent No. 3,854,470 issued in 1974 to Augspurger ("Augspurger 1974"), Ex. 1018, describes the separation of X chromosome containing sperm from Y chromosome containing sperm, followed by freezing of the sperm. Specifically, Augspurger 1974 describes separating X chromosome containing sperm from Y chromosome containing sperm utilizing ion exchange material to differentiate between X chromosome containing sperm and Y chromosome containing sperm. Ex. 1018 (Augspurger 1974), 12:30-13:43. The desired sperm are then removed from the ion exchange material and either "used for insemination or frozen." *Id.*, 13:43-45. Augspurger 1974 also reports that sperm, particularly bovine sperm, can be separated using ion exchange material in combination with centrifugation and pressure differentials. *Id.*, 13:47-14:2. Again, either the X chromosome containing sperm or the Y chromosome containing sperm can be obtained and then "used for insemination or forzen [sic] in a buffer sollution [sic] for later use." *Id.*, 14:2-4.

60. U.S. Patent No. 4,092,229 issued in 1978 to Bhairab Bhattacharya ("Bhattacharya 1978"), Ex. 1019, relates to "a method and apparatus

for controlling the sex of mammalian offspring by separation of X-chromosome female producing sperm of a certain density and electric potential from Y-chromosome male producing sperm of a differing density and electric potential." Ex. 1019 (Bhattacharya 1978), 1:25-30. Bhattacharya 1978 reports that fresh sperm was collected from a male, *id.*, 9:60-61, extended with a "universal medium," *id.*, 9:30-34, and subjected to processes designed to result in the separation of X bearing chromosome containing sperm from Y bearing chromosome sperm, *id.*, 9:67-12:47. Thereafter, the different fractions of sperm were extended to the desired volume and sperm cell number, *id.*, 12:50-53, and equilibrated with glycerol, *id.*, 12:54-56. Bhattacharya 1978 further reports that the sperm "may then be put into one ml. ampoules, sealed, marked as male or female or a mixture in the event of the fraction drawn off from the central convection tube, frozen and stored in liquid nitrogen." *Id.*, 12:56-60. Bhattacharya 1978 further reports that "50 ampules of good-quality, high purity female semen processed as described in the manner of the present invention have been used for biological tests, that is, by insemination and conception and the samples have shown a very high conception rate." *Id.*, 14:39-43.

61. Similarly, U.S. Patent No. 4,083,957 issued to John Lang in 1978 ("Lang 1978"), Ex. 1020, reports that X chromosome bearing sperm obtained from bulls was separated from Y chromosome bearing sperm based on electrical

charge. Ex. 1020 (Lang 1978), 19:1-66, 21:43-54. After extending the sperm subject to such a separation procedure, the sperm was then "sealed in the conventional containers used by cattle artificial inseminators, then cooled and frozen in liquid nitrogen for storage" using "conventional procedures of the art." *Id.*, 20:22-26. Lang 1978 reports that cows were later inseminated with the frozen semen, and the conception rate using the tested spermatozoa was so high as to be "virtually normal." *Id.*, 23:29-33.

62. U.S. Patent No. 4,362,246 ("the '246 patent") issued in 1982 to Adair ("Adair 1982"), Ex. 1021, relates to "an improved method of preserving collected mammalian semen and separating the sperm into X and Y components so that a female of the species may be artificially inseminated with separated semen to produce offspring of a desired and predetermined sex." Ex. 1021 (Adair 1982), 1:7-12. Adair 1982 describes collecting sperm, staining the sperm with a fluorescent dye, feeding the sperm through a particle sorter, detecting the difference in fluorescence between X chromosome containing sperm and Y chromosome containing sperm and separating the sperm. *Id.*, 2:61-4:48. Adair 1982 reports that "[a]fter separation, the sperm may be frozen for use at a future time in a manner which is well known." *Id.*, 4:52-54.

63. In 1982, A. A. Luderer published "Separation of Bovine Spermatozoa by Density on Water Insoluble Newtonian Gels and Their Use for

Insemination," *Biology of Reprod.*, Vol. 26, at 813-824 ("Luderer 1982"), Ex. 1022. In that article, he reports separating bovine sperm into density subpopulations utilizing water insoluble hydrocarbon and silicone gels. Ex. 1022 at 813-814. After separation, fractions of sperm were centrifuged to remove excess fluid, resuspended in a standard extender, cooled, packaged in straws, and frozen. *Id.* at 814. The sperm was later thawed and analyzed for motility and acrosomal integrity, with the author concluding "that top fractions of frozen and thawed spermatozoa retained sufficient motility and intact acrosomal membranes to be used for insemination." *Id.* at 820. Heifers were also inseminated with the frozen separated sperm and, while the desired sex skew of offspring was not achieved, "reasonable pregnancy rates for commercial artificial insemination" were achieved. *Id.* at 821.

64. U.S. Patent No. 4,474,875 issued in 1984 to Shrimpton ("Shrimpton 1984"), Ex. 1023, is predicated on the discovery that "the two sperm genotypes of mammals (X and Y) may be separated according to density characteristics by application of buoyant forces within a liquid separation medium to cause more buoyant sperm to obtain a different level in the separation medium than less buoyant sperm." Ex. 1023 (Shrimpton 1984), 1:51-57. Shrimpton 1984 describes processing sperm "to effect separation of the sperm according to density." *Id.*, 3:25-27. Shrimpton 1984 teaches that, after separation, "the desired

substantially pure sperm fractions should be immediately separated from the top or bottom of the column and preserved for artificial insemination." *Id.*, 9:35-39.

Specifically, Shrimpton 1984 teaches that separated samples of sperm can be, and were, "deep frozen using standard procedures as normally practiced in the artificial insemination industry." *Id.*, 16:38-40. Shrimpton 1984 also discloses that "[a]s needed, sperm samples prepared as above, were removed from cold storage and used in artificial insemination of female mammals. In the specific case of cattle, cows found to be in heat were inseminated with 1 to 2 ccs containing at least 10 to 20 million sperm (before freezing), again using procedures standard in the artificial insemination industry." *Id.*, 16:41-47. Shrimpton 1984 reports that 13 cows were successfully inseminated using separated frozen sperm and that the number of female offspring "represents statistically significant departure from the normal sex ratio in cows." *Id.*, 17:14-15.

65. Beal, White and Garner also reported in 1984 on their efforts to separate and freeze bovine sperm. Ex. 1024 (Beal, W.E., *et al.*, "Sex Ratio after Insemination of Bovine Spermatozoa Isolated using a Bovine Serum Albumin Gradient," 58 J. Animal Sci. 1432-36 (1984) ("Beal 1984")). They reported collecting semen from bulls. *Id.* at 1433. The semen was then extended in a Tris buffer and applied to one or more BSA gradients designed to separate X chromosome bearing sperm from Y chromosome bearing sperm. *Id.* at 1432-1433.

The sperm was then frozen in straws in liquid nitrogen. *Id.* at 1433. After three weeks of frozen storage, the sperm was used to artificially inseminate cows and heifers. Beal 1984 compared the pregnancy rate of the separated and unseparated sperm. *Id.* at 1434. The unseparated sperm resulted in a 61% pregnancy rate, while the sperm that had undergone separation processing resulted in a 65% pregnancy rate. *Id.* at 1434.

66. R. H. Foote also published on the development of fetuses following insemination. Ex. 1025 (Foote, "Normal Development Of Fetuses Resulting from Holstein Semen Processed for Sex Separation," *Theriogenology: An Int'l J. of Animal Reprod.*, Vol. 24, No. 1, at 197-202 (1985) ("Foote 1985")). Foote 1985 reported that "[s]emen from two high fertility Holstein bulls with extensive histories in artificial insemination was specially processed for the purpose of sexing and then frozen." *Id.* at 197. The semen was used to inseminate 200 heifers, resulting in approximately 100 fetuses. *Id.* at 197, 199. Foote 1985 concluded that the "studies support the conclusion that the special processing of the semen did not affect the fetuses" *Id.* at 202.

67. U.S. Patent No. 4,007,087 issued to Ericsson in 1977 ("Ericsson 1997"), Ex. 1026, reports collecting sperm, fractionating the sperm to separate motile sperm from immotile sperm, utilizing standard methods such as centrifugation to concentrate the sperm, freezing the motile sperm using

conventional techniques, and thereafter thawing the frozen separated sperm and using it in artificial insemination. Ex. 1026, 3:59- 9:53.

68. While it is now believed that flow cytometry is the only known method of reliably separating X chromosome containing sperm from Y chromosome containing sperm, in my opinion the above references that describe other methods of separation demonstrate that there is nothing inherent in the process of separating sperm cells that reduces the likelihood that such sperm can thereafter be successfully frozen.

69. Furthermore, as soon as the flow cytometry process had advanced to the point of producing sufficient quantities of sperm for artificial insemination, a person of ordinary skill in the art would have been motivated to freeze separated sperm using known freezing techniques and would have had a reasonable expectation that previously separated sperm could be frozen and maintain viability.

70. Frozen sperm were known to have a variety of benefits, including the ability to ship sperm across the country, and the ability to store sperm for long periods of time. During the pendency of the prosecution of the '265 patent, the '265 patent applicants explained in the prosecution of a related application:

Frozen selected sperm samples which, when thawed, result in viable selected sperm, greatly facilitates the storage and/or shipment of selected sperm samples and allows widespread use of selected sperm. The ease of shipment and resulting availability of the frozen selected sperm samples, enables an increase in production efficiency and production quality of offspring (e.g., livestock, show or race horses, endangered species) having desirable traits.

Ex. 1030 (File History of U.S. Serial No. 09/577,246 ("246 File History") at 113 (Sep. 6, 2001 response at 3). Frozen sperm also allows breeders to take advantage, at the optimal time for the female, of the genetic attributes of high quality bulls located far away from the females to be impregnated. The benefits recited in the '265 patent for frozen separated sperm, *i.e.*, easier storage and shipment and availability of sperm likely to result in offspring with desired traits, are the very same benefits that are achieved by freezing unsorted sperm.

71. And, it was known that the ability to freeze sperm would assist in the widespread application of sorted semen. Ex. 1027 (Polge, "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen, IVF Procedures," in Procs. of the 16th Technical Conf. on Artificial Insemination and Reprod. (1996)), at 10 (reporting that "preliminary experiments have been carried out on freezing sperm after sorting" and that "this option would extend still further

the ability to apply the technology in a variety of breeding programs"). Thus, individuals of ordinary skill in the art would have been motivated to freeze separated sperm.

72. Such individuals also would have had a reasonable expectation of success. Methods of freezing sperm were well known. *See, supra*, at ¶¶ 30-37. Nothing in the art would have suggested that separated sperm could not have been frozen while maintaining viability. While it is true that separated sperm is subject to certain stresses, the same can be said for unseparated sperm, which is subject to the stresses inherent in the collection and freezing process.

IV. THE '265 PATENT

A. Priority Date Of The '265 Patent

73. The '265 patent issued from U.S. Application No. 09/478,299 ("the '299 application"), which was filed on January 5, 2000. Ex. 1001 ('265 patent), at cover. The '299 application claims priority to U.S. Provisional Application No. 60/167,423, filed on November 24, 1999. *Id.*

74. For purposes of this declaration, I have assumed that the priority date for prior art purposes is November 24, 1999, *i.e.*, the earliest filing date of any application to which the '265 patent claims priority.

B. General Overview Of The '265 Patent

75. The title of the '265 patent is "Method of Cryopreserving Selected Sperm Cells." Ex. 1001 ('265 patent), 1:1-2.

76. The field of the invention is "a method for freezing sperm selected for a particular characteristic," and more specifically relates to freezing sperm after separating X chromosome containing sperm from Y chromosome containing sperm. Ex. 1001 ('265 patent), 1:12-16.

1. Background of the invention

77. The '265 patent recognizes that the concept of freezing sperm is not new. Indeed, the '265 patent recognizes in the "Background of the Invention" section that "advances in the cryopreservation and storage of sperm have facilitated widespread distribution and commercialization of sperm intended for artificial insemination or in vitro fertilization." Ex. 1001 ('265 patent), 1:24-27. The '265 patent also recognizes that "techniques for freezing unselected sperm are well known." *Id.*, 2:5-8. The '265 patent also recognizes that "improvements in . . . cryopreservation . . . have enhanced the ability of breeders to produce animals having desired traits." *Id.*, 1:28-31.

78. The '265 patent also recognizes that the concept of separating sperm is not new. Indeed, the '265 patent recognizes in the "Background of the Invention" section that "advances in selection of mammalian sperm based on slight differences in physical characteristics has made it possible to separate sperm based on sex-type, that is, to select for cells containing either the X or Y chromosome. This technique allows the breeder to manipulate the relative percentage of X- or Y-

type sperm in a sample and thereby determine offspring sex." Ex. 1001 ('265 patent), 1:31-35. The '265 patent also recognizes that "improvements in . . . selection . . . have enhanced the ability of breeders to produce animals having desired traits." *Id.*, 1:28-31.

79. The '265 patent also recognizes that "[a] variety of methods are available for selecting cells." Ex. 1001 ('265 patent), 1:44. Flow cytometry is recognized by the '265 patent as "a particularly efficient selection method that has been employed for sorting sperm by sex-type." *Id.*, 1:59-60; *see also id.*, 2:23-24 ("selection using flow cytometry is preferred").

80. The '265 patent suggests that "no technique for cryo-preservation of selected sperm has been described." Ex. 1001 ('265 patent), 2:7-8. The '265 patent also suggests that obtaining viable sperm after selecting and freezing was "surprising because of the well-documented fragility of sperm" and that the inventor was the first to demonstrate that "pregnancies can be achieved with sperm that have been selected and then frozen." *Id.*, 2:48-50, 2:53-55. However, the '265 patent does not contain any evidence supporting these statements. For example, the '265 patent contains no evidence showing that those of ordinary skill were surprised that separating followed by freezing resulted in viable sperm or that others had obtained only unviable sperm after separating and freezing. To the contrary, it was well known in the art that sperm subjected to

separation procedures could be cryo-preserved using techniques known in the art and that such sperm could be used to successfully inseminate females. *See, supra*, at ¶¶ 56-72.

81. Furthermore, the '265 patent does not describe a novel method of freezing sperm. To the contrary, the '265 patent describes methods known in the art and specifies that "any standard freezing method can be employed" to freeze the separated sperm. Ex. 1001 ('265 patent), 10:55-56.

2. The methods described in the specification

82. I below describe the method for freezing separated sperm set forth in the specification.

83. The first step in the method described in the "Detailed Description of the Invention" section of the '265 patent is obtaining a selected sperm sample. Ex. 1001 ('265 patent), 3:54-61. The '265 patent defines "selection" as "a method whereby a sample is subdivided based on presence or absence of a specific characteristic." *Id.*, 3:19-22. The '265 patent defines a "selected sperm sample" as "a sample obtained by subjecting a source sample to selection for the specific characteristic" and notes that a "selected sperm sample is therefore enriched, relative to the source sample, in sperm having the specific characteristic." *Id.*, 3:22-26.

84. The '265 patent notes that "[s]perm from any species can be selected and frozen according to the method of the invention." Ex. 1001 ('265 patent), 3:67-4:2. The specification indicates that "[h]uman sperm, bovine, equine, porcine, ovine, elk, and bison sperm are particularly preferred." *Id.*, 4:4-7.

85. There is nothing novel about this step, and the '265 patent notes that a "wide variety of methods for selecting cells from a mixed populations [sic] are available, including, for example, selection based on binding of cells or cell components with antibodies, antibody fragments, or other binding partners and differential staining." Ex. 1001 ('265 patent), 4:15-19. The '265 patent also lists a variety of other selection methods, particularly for sex-selected sperm, that were known, including magnetic techniques, columnar techniques, gravimetric techniques, techniques based on differences in electrical properties, techniques based on differences in both electrical and gravimetric properties, techniques based on differences in motility, and others. *Id.*, 4:20-39. The preferred method is identified as flow cytometry. *Id.*, 4:40-41.

86. Selecting sperm by flow cytometry is described in, for example, U.S. Patent No. 5,135,759 to Johnson, which issued in 1992 and provides a summary of how sperm cells can be sorted using flow cytometry. Ex. 1008 (Johnson 1992). Flow cytometry allows sperm to be selected based on DNA content. *Id.*, 2:47-49, 2:64-67. Specifically, cells are first dyed with a nontoxic

DNA stain, such as Hoechst bisbenzimidazole 33342 fluorochrome. *Id.*, 4:27-29. The sperm are then passed through a laser beam and the flow cytometer "measures the amount of fluorescent light given off when the sperm, previously treated with a fluorescent dye, pass through a laser beam." *Id.*, 3:4-6. "Because the X chromosome contains more DNA than the Y chromosome, the female sperm (X) takes up more dye and gives off more fluorescent light than the male sperm." *Id.*, 3:10-13.

87. The '265 patent next describes cooling of the selected sperm sample. Ex. 1001 ('265 patent), 3:54-61. The '265 patent explains that sperm is preferably cooled from about 22° C to about 5° C over a period of about 60 minutes to 24 hours, most preferably over a period of about 90 to 120 minutes. *Id.*, 7:59-65. There is nothing novel about this cooling step, and the '265 patent explains that "[c]ooling can be accomplished by any convenient method, including simply placing the selected sperm sample in a 5° C[elsius] environment." *Id.*, 7:65-67.

88. The third step in the method described in the '265 patent is isolating sperm from the selected sperm sample. Ex. 1001 ('265 patent), 3:54-61. There is nothing novel about this isolation step, and the '265 patent explains that "[a]ny of a variety of methods suitable for recovering cells from a suspension can

be used to isolate the sperm, including for example, filtration, sedimentation, and centrifugation." *Id.*, 8:10-24.

89. The fourth step in the method described in the '265 patent is adding final extender to the isolated sperm to produce a suspension of sperm. Ex. 1001 ('265 patent), 3:54-61. The '265 patent defines an extender as "any medium that tends to preserve sperm viability." *Id.*, 3:30-31. The '265 patent further defines an "initial extender" as "a medium used to extend sperm prior to the isolation step of the method" and a "final extender" as "a medium used to extend sperm prior to the freezing step of the method" *Id.*, 3:34-38. The '265 patent explains that the "initial extender" and the "final extender" can be the same or different. *Id.*, 8:34-36. The '265 patent also makes clear that the extender can be added as a single mixture or component-by-component. *Id.*, 5:31-37 ("If no extender components are present, an extender or extender fraction is typically added to the selected sperm sample before the sperm are isolated from the sample. If only some extender components are present, additional components can optionally be added so that selected sperm sample includes a complete extender or an extender fraction before the isolation step.").

90. Again, there is nothing novel about this step, and the '265 patent explains that a variety of extenders were known in the prior art. For example, the '265 patent notes that two step extenders (where one fraction contains glycerol and

the other does not) were "well known." Ex. 1001 ('265 patent), 5:22-30. The '265 patent also explains that "[o]ther extenders standardly used to freeze sperm can also be employed as the final extender in freezing selected sperm. A variety of extenders optimized for use in freezing sperm from various species have been described, and many are commercially available." *Id.*, 10:18-28.

91. The '265 patent explains that the extender should include a physiologically acceptable carrier and "commonly" includes a cryoprotectant, a component that maintains osmolality and buffers pH, an organic substance that prevents cold shock, an energy source, and/or one or more antibiotics. Ex. 1001 ('265 patent), 5:50-62.

92. The '265 patent notes that glycerol is commonly used as a cryoprotectant for human and bovine sperm and notes that other cryoprotectants are "well known for many commercially valuable sperm and can readily be determined empirically for other types of sperm." Ex. 1001 ('265 patent), 6:3-12.

93. The '265 patent also states that "[s]ubstances helpful in maintaining omolality [sic] and pH within these [desired] ranges are well known in the art" Ex. 1001 ('265 patent), 6:22-24. Although the selection of a buffer can vary depending on other components in the extender and the species of sperm, the '265 patent reports that "selection of such a component for use in the present invention is, however, within the level of skill in the art." *Id.*, 6:30-36.

94. Organic substances that prevent cold shock are also, according to the '265 patent, "well known" in the art. Ex. 1001 ('265 patent), 6:38-42. Suitable substances include disaccharides, trisaccharides, egg yolk and milk. *Id.*, 6:48-52.

95. The '265 patent also explains that, "[a]s is well known in the art, sugars, particularly monosaccharides, provide a convenient energy source" Ex. 1001 ('265 patent), 6:66-7:2.

96. The '265 patent also makes clear that it was known to include antibiotics in an extender. As that patent explains, "[a]ny of a variety of antibiotics useful in the cryopreservation of cells can also be employed in the extender." Ex. 1001 ('265 patent), 7:19-34.

97. The fifth step in the method described in the '265 patent is freezing the sperm. Ex. 1001 ('265 patent), 3:54-61. Again, as the '265 patent expressly explains, freezing sperm is not novel. To the contrary, the '265 patent reports that "any standard freezing method can be employed, provided the freezing rate is not too rapid" *Id.*, 10:55-57.

98. Although the '265 patent notes that freezing should not be in excess of about 0.5° C per minute, Ex. 1001 ('265 patent), 10:55-57, I note that I am unaware of any methods of freezing sperm in which the sperm is frozen at such a slow rate. The '265 patent also provides "an exemplary, preferred embodiment"

in which the sperm is frozen in three stages at rates of 40 to 65° C per minute until reaching about -15° C, 25 to 35° C per minute until reaching about -60° C, and then plunged into liquid nitrogen at about -100° C. *Id.*, 10:57-67. Elsewhere, the '265 patent states that sperm were "frozen conventionally." *Id.*, 17:17. Thus, I suspect the reference to a freezing rate of 0.5° C per minute or less is likely an error.

99. In short, the '265 patent explains that any known method of separating X chromosome containing sperm from Y chromosome containing sperm and any known method of freezing sperm can be utilized in the claimed method of freezing separated sperm cells. In my opinion, and as I discuss in greater detail below, nothing novel or nonobvious is described or claimed in the '265 patent.

C. Prosecution History Of The '265 Patent

100. I understand that the '265 patent issued from U.S. Application No. 09/478,299 filed on January 5, 2000. Ex. 1001 ('265 patent) at cover. I further understand that the '265 patent also claims priority to a provisional application, No. 60/167,423, filed on November 24, 1999. *Id.*

101. I note that, during the prosecution history of the '265 patent and the related applications, the applicant repeatedly – and in my view incorrectly – informed the PTO that the named inventor was the first to obtain viable separated, frozen sperm in order to overcome prior art rejections. *See, e.g.*, Ex. 1002 (File

History of U.S. Patent No. 7,208,265 File History ("265 File History")) at 221 (June 25, 2001 response at 3) ("Although techniques for freezing unselected sperm are known, *no technique for repeatedly producing viable cryopreserved selected sperm has been described prior to the instant disclosure.*" (emphasis in original); "*The present inventors have demonstrated, for the first time, that pregnancies can be achieved with sperm that have been selected and then frozen and that the resulting offspring exhibit the selected characteristic.*") (emphasis in original); Ex. 1030 ('246 File History) at 114 (Sep. 6, 2001 response at 4) ("Moreover, prior to applicant's invention, no viable pregnancies had been achieved or no offspring exhibiting the selected characteristic had been produced using selected cryopreserved sperm."). As discussed herein, *see supra* at ¶¶ 30, 56-72, others had reported freezing separated sperm, using that frozen sperm in artificial insemination, and successfully achieving pregnancies. Therefore, to the extent that the Patent Office allowed the claims to issue based on the representation that the application was the first to obtain viable, separated, frozen sperm, the Patent Office erred in my opinion.

D. Claim Construction

102. I understand that in the context of an IPR proceeding, claims are given their broadest reasonable interpretation in light of the specification.

103. I understand that, where a patent applicant provides an explicit definition of a claim term in the specification, that definition controls the interpretation of that term in the claim.

104. I also understand that if no explicit definition is provided for a term in the patent specification, the claim term must be given its plain meaning unless that meaning is inconsistent with a meaning given to the term by its use in the specification and the claims.

1. "sorting" sperm cells

105. Claim 1 is directed to a method of freezing "sorted" sperm cells and recites the step of "sorting" sperm cells using flow cytometry or fluorescence activated cell sorting.

106. The specification of the '265 patent expressly defines the term "sorting" as "a selection method carried out using a fluorescence-activated cell sorter (FACS)." Ex. 1001 ('265 patent), 3:27-29. The specification of the '265 patent also expressly defines "selection" to mean "a method whereby a sample is subdivided based on presence or absence of a specific characteristic (unless context dictates otherwise)." *Id.* 3:19-22. These definitions are consistent with the plain and ordinary meaning of "sort" in the present context, which is "to separate from others." Ex. 1028 at 1299 (The American Heritage College Dictionary, 3d Ed. (1997)). A person of ordinary skill in the art would understand that "sorting"

sperm cells requires that one population of cells be set apart from another population of cells, *e.g.*, that X chromosome bearing sperm be set apart from Y chromosome bearing sperm.

107. The specification of the '265 patent uses the terms “sort” and “sorting” consistent with these accepted meanings. The '265 patent specification states that: "advances in selection of mammalian sperm based on slight differences in physical characteristics has made it possible to separate sperm based on sex-type, that is, to select for cells containing either the X or Y chromosome." Ex. 1001 ('265 patent), 1:31-35. The specification also defines "selection" as "a method whereby a sample is subdivided based on presence or absence of a specific characteristic" and notes that a "selected sperm sample is therefore enriched, relative to the source sample, in sperm having the specific characteristic." *Id.*, 3:19-26.

108. The specification also notes that "flow cytometry" is a preferred method for "separating cells" and specifically cites to two patents by Johnson that describe flow cytometry. Ex. 1001 ('265 patent), 4:40-59.

109. A person of ordinary skill in the art would understand that flow cytometry results in two discrete populations of cells, typically a population of X chromosome bearing sperm and a population of Y chromosome bearing sperm. *See, supra*, at ¶¶ 50-26.

110. Thus, it is my opinion that a person of ordinary skill would understand the term "sorting" to mean "separating."

2. "without the presence of protective compounds in seminal plasma"

111. Claim 1 of the '265 patent included the term "without the presence of protective compounds in seminal plasma." The '265 patent does not provide an express definition of what this means. The plain and ordinary meaning of "without" is "[w]ith something absent or lacking" or "[n]ot having; lacking" or "[n]ot accompanied by; in the absence of." Ex. 1028. The '265 patent describes in Example 1B the process of centrifuging the sperm and removing the supernatant to obtain a sperm pellet. In my opinion, a skilled person would recognize that this process would result in removing nearly all of the seminal plasma. Ex. 1001 ('265 patent), 13:6-10. Thus, the term "without the presence of protective compounds in seminal plasma" should at least cover this technique of removing nearly all of the seminal plasma through centrifugation. This is also in accord with the plain and ordinary meaning of the words in the claim.

3. "based on sex-type to provide a collection of sex-selected sperm cells obtained using flow cytometry or fluorescence-activated cell-sorting"

112. Claim 1 of the '265 patent includes the term "based on sex-type to provide a collection of sex-sorted sperm cells obtained using flow cytometry or fluorescence-activated cell-sorting." This term would have been well understood

by those of ordinary skill in the art. The patent notes that flow cytometry is a preferred method for separating cells based on differential staining with fluorescent dyes. Ex. 1001 ('265 patent), 4:40-43. In addition, the patent states that: "In fluorescence activated cell sorting ('FACS'), the cells are 'sorted' into different populations based on the fluorescence intensity upon irradiation. *Id.*, 4:43-46. The '265 patent credits Johnson for employing this strategy for sex-selection of sperm. *Id.*, 4:43-54. A person of ordinary skill in the art would understand that flow cytometry results in two discrete populations of cells, typically a population of X chromosome bearing sperm and a population of Y chromosome bearing sperm. Accordingly, a person of ordinary skill in the art would understand the term to require the "sorting" be based on sex-type to provide a collection of sex-selected sperm cells and that the sorting be accomplished using either of the recited techniques, which were well known.

4. "extender"

113. The specification of the '265 patent expressly defines "extender" as "any medium that tends to preserve sperm viability" and "extension" is defined to mean "the dilution of sperm with extender." Ex. 1001 ('265 patent), 3:30-32. Thus, in my opinion, a person of ordinary skill in the art would agree with the express definition found in the specification and understand the term "extender" to mean: "any medium that tends to preserve sperm viability."

5. “a concentration of sperm cells of about 5 million per milliliter of extender to about 10 million per milliliter of extender”

114. Claim 1 of the '265 patent requires a concentration of sperm cells, after sorting and prior to freezing, of "about 5 million per milliliter of extender to about 10 million per milliliter of extender." The '265 patent does not provide an express definition for the term "about" in this limitation. However, I understand that the '265 patent applicants added the limitation during prosecution and argued that Table 1 in Example 1A provided support for the values. Ex. 1002 ('265 File History) at 422, 449-450, 746-747 (Feb. 28, 2005 Response at 9; Nov. 14, 2005 Response at 9-10; Aug. 9, 2006 Response at 10-11). One of ordinary skill in the art would understand that numerical limitations encompass a variance to take into account measurement tolerances associated with determining sperm concentrations. The typical methodologies to determine sperm cell concentrations varied by up to plus or minus 5-10% with sperm concentrations in the 5 to 10 million range. Accordingly, under a broadest reasonable interpretation, the cell concentrations measures may vary by up to 10%. Under these circumstances, one would understand "about 5 million" to encompass 4.5 to 5.5 million sperm per milliliter and "about 10 million" to encompass 9 to 11 million sperm per milliliter.

6. "freezing"

115. Claim 1 is directed to a method of "freezing" separated sperm cells and recites the step of "freezing" a sperm sample.

116. The plain and ordinary meaning of "freeze" is to "pass from the liquid to the solid state by loss of heat" or to "preserve (foods, for example) by subjecting to freezing temperatures." Ex. 1028 (The American Heritage College Dictionary, 3d Ed. (1997)) at 543-544.

117. The specification of the '265 patent is in accord with the plain and ordinary meaning. The '265 patent notes that "any standard freezing method can be employed, provided the freezing rate is not too rapid." Ex. 1001 ('265 patent), 10:55-57.

118. Although the specification suggests in one place that a rate that is not in excess of about 0.5° C/minute is preferred, Ex. 1001 ('265 patent), 10:55-57, the specification also provides "an exemplary, preferred embodiment" in which a freezing rate far in excess of 0.5° C per minute is used:

[T]he sperm are placed in static liquid nitrogen vapor, and freezing is carried out in three distinct stages over a period of about 10 minutes. In the first stage of freezing, the sperm are cooled from about 5° C. to about -15° C. at a rate of about 40° C./minute to about 65° C./minute. In the second stage of freezing, the sperm are cooled from about -15° C. to about -60° C. at a rate of about 25°

C./minute to about 35° C./minute. In the third stage, the sperm are plunged into liquid nitrogen at about -100° C.

Id., 10:58-67.

119. Elsewhere in the examples, the '265 patent refers to sperm "frozen conventionally" or "frozen in static liquid nitrogen vapor." Ex. 1001 ('265 patent), 17:17; *see also id.*, 19:28. In my view, a person of ordinary skill in the art would understand that any rate of freezing that resulted in viable sperm was acceptable and would not read a specific rate into the claims.

120. Thus, in my opinion, a person of ordinary skill in the art would understand "freezing" to refer to any method for taking sperm from a liquid to a solid state by loss of heat, so long as the cells retains sufficient viability to result in pregnancy.

V. PATENTABILITY ANALYSIS OF THE '265 PATENT

A. The Claims

121. Claim 1 of the '265 patent is reproduced below:

1. A method of cryopreserving sex-selected sperm cells, comprising:
 - a. obtaining sperm cells from a species of a non-human male mammal;
 - b. sorting said sperm cells, without the presence of protective compounds in seminal plasma, and based upon

sex-type to provide a collection of sex-selected sperm cells obtained using flow cytometry or fluorescence-activated cell sorting;

c. cooling said sex-selected sperm cells;

d. suspending said sex-selected sperm cells in an extender to provide a concentration of sperm cells of about 5 million per milliliter of extender to about 10 million per milliliter of extender; and

e. freezing said sex-selected sperm cells in said extender.

122. The challenged dependent claims include additional limitations, as set forth in the claims.

B. Detailed Description Of Selected Prior Art

123. For the purposes of the present analysis, I have limited the discussion of my unpatentability analysis to a handful of specific references, which are described in detail below. These references are by no means the only references which I believe demonstrate that claims 1-4, 8-20, 22 and 26-28 of the '265 patent are unpatentable. Any number of references, including but not limited to many of the publications discussed and cited in the background section above, could have been applied to claims 1-4, 8-20, 22 and 26-28 to reach a conclusion of unpatentability.

1. Detailed description of Seidel 1997

124. U.S. Patent No. 7,195,920 issued in 2007 to George Seidel, Lisa Herickhoff and John Schenk, and claims priority back to an application filed on December 31, 1997 ("Seidel 1997"). *See* Ex. 1005 (Seidel 1997) at cover. As such, I understand it qualifies as prior art to the '265 patent pursuant to 35 U.S.C. § 102(e) (2010).

125. Seidel 1997 is entitled "Collection Systems for Cytometer Sorting of Sperm." Ex. 1005 (Seidel 1997), 1:1-2.

126. Seidel 1997 relates to "the field of sex selection in mammalian offspring" and "is especially relevant to the aspect of low dose artificial insemination for creating the desired sex of offspring." Ex. 1005 (Seidel 1997), 1:12-15.

127. Seidel 1997 discloses a process in which semen is collected. For example, in the context of bovine insemination, semen is collected from the bull through the use of an artificial vagina. Ex. 1005 (Seidel 1997), 13:17-21.

128. Seidel 1997 notes that antibiotics may be added to the sperm. Ex. 1005 (Seidel 1997), 13:25-26.

129. Seidel 1997 discloses that the collected sperm is thereafter stained with a fluorescent dye. Ex. 1005 (Seidel 1997), 13:35-52. In the examples, the dye used is Hoechst 33342. *Id.*, 15:21-24.

130. Seidel 1997 discloses that the sperm can then be separated into X chromosome containing sperm cells and Y chromosome containing sperm cells. Ex. 1005 (Seidel 1997), 13:56-58.

131. Seidel 1997 discloses that "[m]any methods have been attempted to achieve the separation of X- and Y-chromosome bearing sperm" but that "the only quantitative technique used to achieve the separation of X- and Y-chromosome bearing sperm has been that involving individual discrimination and separation of the sperm through the techniques of flow cytometry." Ex. 1005 (Seidel 1997), 1:39-40, 1:62-66. Seidel 1997 discloses that separation using flow cytometry "appeared possible as a result of advances and discoveries involving the differential dye absorption of X- and Y-chromosome bearing sperm." *Id.*, 1:66-2:1.

132. Seidel 1997 explains that flow cytometry is a technique "which is well understood." Ex. 1005 (Seidel 1997), 5:45-47. Figure 1 of Seidel 1997 depicts a flow cytometer. *Id.*, 5:55-56, Fig. 1. Example 1 in Seidel 1997 refers to the use of a MoFlo® flow cytometer. *Id.*, 15:24-27

133. Seidel 1997 explains that the cell sensing system contained within the flow cytometer can "cause an action depending upon the relative presence or relative absence of a particular dye which may be excited by some stimulant such as the laser exciter." Ex. 1005 (Seidel 1997), 6:15-24. Seidel 1997

further explains that "the differing length of the X-chromosome and the Y-chromosome causes different levels of staining[.] Thus, by sensing the degree of dye present in the sperm cells it is possible to discriminate between X-bearing sperm and Y-bearing sperm by their differing emission levels." *Id.*, 6:24-30.

134. Seidel 1997 also explains that "by sensing some property of the cells or other items the flow cytometer can discriminate between cells based on a particular characteristic and place them in the appropriate collector." Ex. 1005 (Seidel 1997), 6:42-45. In other words, X chromosome containing cells are deflected in one direction and caught in one container, while Y chromosome containing cells are deflected in another direction and caught in another container. *Id.*, 6:45-51.

135. After the sperm has been separated, Seidel 1997 discloses that the sperm can be concentrated to about 3 to 5 million sperm cells per ml through the use of centrifugation to remove fluid. Ex. 1005 (Seidel 1997), 13:61-64.

136. Seidel 1997 discloses that the concentrated sperm can then be extended using either a 20% egg yolk citrate extender or Cornell Universal Extender or the like. Ex. 1005 (Seidel 1997), 13:64-66. I read "or the like" to mean that other extenders may be used.

137. Seidel 1997 discloses that Cornell Universal Extender may contain sodium citrate dehydrate, NaHCO_3 , potassium chloride, glucose, glycine, citric acid and egg yolk. Ex. 1005 (Seidel 1997), 13:66-14:10.

138. Seidel 1997 also discloses that the extender may contain antibiotics, such as tylosin, gentamicin and linco-spectin. Ex. 1005 (Seidel 1997), 12:6-11.

139. Seidel 1997 discloses that after the last extension, there may be 3 to 5 million sperm per ml. Ex. 1005 (Seidel 1997), 14:11-12.

140. Seidel 1997 discloses that the "sample may then be cooled to slow the sperm's metabolism and to permit use over longer periods of time." Ex. 1005 (Seidel 1997), 14:12-14. Seidel 1997 discloses cooling the sperm to 5° C over 75 minutes. *Id.*, 15:37-39.

141. Seidel 1997 discloses that, if desired, the sperm can be further processed to result in a dose of 300,000 sperm per 0.184 ml. Ex. 1005 (Seidel 1997), 14:16-22.

142. Seidel 1997 also discloses that the "sperm cell specimen may then be placed in a straw for use in artificial insemination and may be transported to the cows or heifers to be inseminated." Ex. 1005 (Seidel 1997), 14:26-28.

143. Example 1 of Seidel 1997 demonstrates that separated sperm are viable. Specifically, Example 1 reports the use of X chromosome containing

sperm separated using a MoFlow® flow cytometer/cell sorter in artificial insemination and notes that the pregnancy rate of heifers inseminated with separated semen was not significantly different than the rate achieved with unseparated semen. Ex. 1005 (Seidel 1997), 15:16-16:9.

2. Detailed description of Salisbury

144. Salisbury is (and was), in my experience, one of the most respected texts in the field of artificial insemination of cattle and gives a detailed description of the state of the art as of the time it was published. In fact, I obtained a copy of the second edition of Salisbury shortly after it was published in 1978.

Ex. 1006. Individual chapters are written by respected experts in the field.

145. Salisbury contains an entire chapter, chapter 17, devoted to "Principles and Techniques of Freezing Spermatozoa." Ex. 1006 (Salisbury) at 494-554.

146. Salisbury notes that since the discovery that glycerol protected sperm, "freezing has been the preferred method of preserving spermatozoa."

Ex. 1006 (Salisbury) at 494.

147. Salisbury contains several pages devoted to the types of extenders commonly used when freezing sperm. Ex. 1006 (Salisbury) at 498-507.

148. Salisbury explains that the basic components of extenders for freezing bull sperm are: (1) water; (2) dissolved ionic and nonionic substances to

maintain osmolality and to buffer the pH of the medium; (3) organic materials with the capacity to prevent cold shock, such as egg yolk or milk; (4) cryoprotective agents such as glycerol; (5) simple sugars for an energy source or disaccharides and trisaccharides as added cryopreservatives; (6) additives such as enzymes that may improve fertility; and (7) antibiotics to control microbial growth. Ex. 1006 (Salisbury) at 498-99.

149. Salisbury teaches that a variety of buffers can be used, including sodium citrate dihydrate and tris(hydroxymethyl) amino methane. Ex. 1006 (Salisbury) at 499. Salisbury notes that "yolk-citrate remains the most widely used seminal extender." *Id.* However, Salisbury also notes that tris-buffered egg yolk extenders provide "excellent buffering capacity" and are suitable extenders at temperatures ranging from 25° C to -196° C. *Id.* at 455. Salisbury also notes that it "is clear that Tris types of organic buffers provide a versatile series of solutions that can be used to formulate desirable semen extenders" and "are suitable for" frozen semen. *Id.* at 457.

150. Salisbury reports that the optimal pH for a seminal extender is in the range of 6.5-7.5, depending upon the ions in the extender. Ex. 1006 (Salisbury) at 502. In particular, the optimal pH for citrate extender is 6.5 to 7.0. *Id.*

151. Salisbury teaches that egg yolk is "a valuable constituent in preserving fertility." Ex. 1006 (Salisbury) at 499.

152. Salisbury teaches that glycerol "is the most widely used cryopreservative for bull spermatozoa." Ex. 1006 (Salisbury) at 500. Salisbury also teaches that "[g]lycerol for the freezing of bull semen remains the standard of comparison for any cryoprotective agent" and that "[n]o substitute has yielded results as satisfactory." *Id.* at 498. Salisbury teaches that glycerol is added as part of the final extender. *Id.* at 507 ("The partially diluted semen is then glycerolated by the additional of an equal volume of extender that contains twice the final desired concentration of glycerol."). Salisbury notes that when sperm is frozen by conventional methods in glass ampoules, approximately 7% glycerol is optimal for citrate-yolk and Tris-yolk extenders. *Id.* at 500. Salisbury also, however, refers to such extenders with about 6% glycerol. *Id.*

153. Salisbury explains that monosaccharides (*i.e.*, simple sugars) such as glucose and fructose are sugars from which sperm may derive energy. Ex. 1006 (Salisbury) at 501.

154. Salisbury explains that microbial contamination of semen may: (1) directly affect the sperm; (2) compete for substrate in an extender; and (3) infect inseminated females, resulting in lower conception rates and increased embryonic mortality or abortion. Ex. 1006 (Salisbury) at 467. Accordingly,

"[c]hemical treatment of semen to inhibit multiplication of these contaminants or to kill them without damaging the sperm cells is therefore important." *Id.* at 467-68. Salisbury teaches that "[e]ffective agents" that were "easy to use," "would control bacterial growth," and be "nontoxic to sperm" include penicillin and streptomycin, among others. *Id.* at 469-72. Salisbury explains that "penicillin and streptomycin have been widely used since about 1950" and that their "popularity stems from the overwhelming evidence of their beneficial effects on fertility." *Id.* at 470. Salisbury also teaches that lincomycin and spectinomycin have been used in combination to control bacteria. *Id.* at 470-72.

155. Salisbury teaches that sperm should be cooled before freezing "for maximum livability of the cells." Ex. 1006 (Salisbury) at 509. Salisbury teaches that sperm can be cooled to 5° C and specifically notes that studies have shown that sperm revival was greater when sperm was cooled to 5° C prior to glycerol addition than when it was only cooled to 20° C. *Id.* at 510-11. According to Salisbury: "Cold shock occurs when bovine spermatozoa are subjected to sudden cooling." *Id.* at 508. Salisbury recommends slow cooling to avoid deleterious effects of "cold shock." *Id.* at 509. More specifically, Salisbury reports that post-thaw motility was maintained significantly longer in semen that was cooled 4 hours before freezing than in semen cooled only 1 hour. *Id.* at 510.

156. Salisbury reports that investigators have "demonstrated that post-thaw motility and fertility of bovine spermatozoa are both enhanced by extending the interval between adding the glycerol and beginning freezing." Ex. 1006 (Salisbury) at 514. Salisbury notes that this stage of semen processing is referred to as "glycerol equilibration." *Id.* Salisbury notes that studies "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period" and reports numerous results of experiments designed to measure the influence of glycerol equilibration, and recommends that sperm be equilibrated for between 4 to 18 hours. *Id.* at 514-15.

157. Salisbury also teaches that sperm can be packaged in ampoules, straws or pellets prior to freezing. Ex. 1006 (Salisbury) at 516-19. The data reported by Salisbury included sperm concentrations of 5-10 million sperm per milliliter prior to freezing. *Id.* at 517-18. For example, Salisbury reports data from a study indicating that successful pregnancies were obtained after insemination with 0.375×10^6 , 0.75×10^6 and 3×10^6 live sperm after thawing. *Id.* at 518, citing Jondet, R., "Contribution to the assessment of the minimal number of frozen spermatozoa necessary to obtain fertilization in the cow," 7th Inter. Congr. Animal Reprod. And Artif. Insem., Munich, II: 1143-1148 (1972), Ex. 1034 (Jondet 1972). The cited study (Jondet 1972) reports the "total sperm number per

dose before freezing” and also the volume of the doses in the study.¹ *Id.* at 1447-1448. From this information, it is simple to calculate the sperm concentrations in the study prior to freezing. For example, Jondet used 4,687,000 total sperm from bull “A” prior to freezing in a 0.6 ml straw. *Id.* at 1447. This equates to a pre-freezing concentration of 7.8 million sperm per milliliter (4,687,000 total sperm/0.60 ml). This was used to achieve a conception rate of 68.77%. *Id.* at 1448. By way of further example, Jondet used 1,127,819 total sperm from bull “A” prior to freezing in a 0.2 ml straw. *Id.* at 1447. This equates to a pre-freezing concentration of 5.6 million sperm per milliliter (1,127,819 total sperm/0.20 ml). This was used to achieve a conception rate of 54.54%. *Id.* at 1448.

158. Salisbury also teaches that there is a wide range of sperm concentrations that can be used successfully to inseminate animals such as cattle. Ex. 1006 (Salisbury) at 476-78. Specifically, Salisbury notes that one study showed that insemination with 4 million motile sperm "gave initial high fertility" and that when the number was decreased to 2 million "fertility changed little." *Id.* at 477. Even insemination with as few as 500,000 unfrozen sperm was shown to

¹ In general, about 50% of sperm are killed or rendered immotile during freezing and thawing. Ex. 1006 (Salisbury) at 495. However, Jondet 1972 provides the actual number of sperm before and after freezing. Ex. 1034 (Jondet) at 1147.

result in 32% and 46% conception. *Id.* In addition, as discussed above, Salisbury reports that post-frozen motile sperm doses as low as $.375 \times 10^6$ and $.75 \times 10^6$ were successfully used to inseminate cows. Ex. 1006 (Salisbury) at 518, citing Ex. 1034 (Jondet 1972).

3. Detailed description of Spaulding

159. Spaulding describes a method to separate living sperm cells using flow cytometry. Ex. 1031, 4:24-34; 7:43-10:9. Spaulding also describes a different method of sorting sperm cells, but that method is distinct from the flow cytometry method. Ex. 1031 (Spaulding), 4:24-34 ("The present invention also provides a method to separated living cells based on DNA content. The method involves separating the cells by flow cytometry, the cytometer having been improved substantially to increase its ability to recognize fine distinctions in fluorescence."). Specifically, Spaulding describes the flow cytometry method of sorting sperm in Example 1. Example 1 describes the "preparation of the sperm" prior to sorting. Ex. 1031 (Spaulding), 9:15-36. As part of the preparation, the sperm was washed three times and then centrifuged to remove the seminal plasma. *Id.* col 9:25-27 ("We centrifuged the cells at 483Xg for 20 minutes and resuspended them to remove seminal plasma proteins."). This preparation process would have removed all, or nearly all, of the seminal plasma prior to sorting. Spaulding reports that: "The objective was to match the refractive indices between

sheath and sample fluid." *Id.*, 9:35-36. After removal of the seminal plasma and addition of the dye, the sperm was then sorted using a flow cytometer. *Id.*, 9:38-10:8. After sorting, two viable subpopulations enriched for X- or Y- sperm, respectively, were obtained. *Id.*, 9:65-66.

160. I understand that the Examiner allowed the '265 patent claims on November 5, 2006 citing in the reasons for allowance that "the declaration of Dr. Schenk filed January 20, 2006, proves that [Spaulding was] inoperable and thus cannot be used in a 35 U.S.C. 103 rejection against the claims." Ex. 1002 ('265 File History) at 783 (Dec. 5, 2006 Notice of Allowability at 3). I have reviewed the Schenk declaration. In my opinion, the statements in the declaration about inoperability in the Spaulding reference relate to the technique of using antibodies as a way of discriminating between X- and Y- bearing sperm cells, not the sorting of sperm by flow cytometry to obtain viable sperm of Example 1. *See* Ex. 1002 ('265 File History) at 496-497 (Jan. 5, 2000 Schenk Decl. at ¶ 14).

4. Detailed description of Fugger

161. E.F. Fugger, S.H. Black, K. Keyvanfar and J.D. Schulman published "Births of normal daughters after MicroSort sperm separation and intrauterine insemination, in-vitro fertilization, or intracytoplasmic sperm injection," in Vol. 13, No. 9 of Human Reproduction ("Fugger"), Ex. 1007. The

article was published in September 1998, more than a year before the filing date of any application from which the '265 patent claims priority. *Id.* at 2367.

162. The article was published in 1998 and received by at least one library by September 24, 1998. Ex. 1007 (Fugger) at cover.

163. Fugger reports that "[b]ased on the birth of over 400 normal offspring from four species of animals," a "trial was established to assess flow cytometric separation of X- and Y-bearing spermatozoa in human subjects." Ex. 1007 (Fugger) at 2367. Individuals entered the trial for prevention of sex-linked disorders or for family balancing. *Id.*

164. Fugger reports that "[f]resh or frozen semen specimens were provided for sorting." Ex. 1007 (Fugger) at 2367.

165. Sperm to be sorted was "extended, centrifuged, resuspended, [and] filtered," which would result in removal of seminal plasma. Ex. 1007 (Fugger) at 2367. The paper by Johann *et al.* cited by Fugger for this step further describes the centrifugation conditions, which would have resulted in removal of all, or nearly all, of the seminal plasma. *See* Ex. 1032 (R. Johann, R.S. Jeyendran, J.P.W. Vermedien, L.J.D. Zaneveld, "Human sperm selection by glass wool filtration and two-layer, discontinuous Percoll gradient centrifugation," Vol. 51, No. 4, Fertility and Sterility (1989) ("Johann 1989")) at 686. In particular, Johann 1989 describes the sequential centrifugation, removal of supernatant and

resuspension of the sperm, three times, and specifies the centrifugation conditions of each (750 x g for 15 minutes, 200 x g for 5 minutes and 200 x g for 5 minutes).

Ex. 1032 (Johann 1989) at 686.

166. Sperm were treated with a solution of Hoechst 33342, a fluorochrome bisbenzimidazole, using known techniques. Ex. 1007 (Fugger) at 2367.

167. The sperm were then sorted by flow cytometry as described in Johnson et al., "Gender preselection in humans? Flow cytometric separation of X and Y spermatozoa for the prevention of X-linked diseases," 8 Human Reprod. 1733-79 (1993). Ex. 1007 (Fugger) at 2367. Fugger reports that flow cytometry is based on differences in the amount of DNA between X chromosome bearing sperm and Y chromosome bearing sperm and notes that X chromosomes are larger than Y chromosomes. *Id.*

168. Fugger reports that sperm were sorted using a buffered fluid with either a modified Epics® 753 or a modified fluorescence-activated cell sorter. Ex. 1007 (Fugger) at 2367-2368. Fugger also reports that "[f]luorescence emitted from each sperm cell was detected through a 400-nm long pass filter" *Id.* at 2368.

169. Fugger reports that the "enriched fraction of the sorted sample was collected." Ex. 1007 (Fugger) at 2367-2368.

170. Thereafter, sorted specimens were extended with TEST yolk buffer freezing medium from Irvine Scientific. Ex. 1007 (Fugger) at 2368. While the recipe for the TEST yolk buffer is not expressly provided, it was known by those of ordinary skill in the art that TEST yolk buffer for freezing sold by Irvine Scientific typically contains sodium citrate, TES buffer, trizma base, egg yolk, fructose, gentamicin (an antibiotic) and glycerol. Ex. 1029 (Catalog No. 90128; Irvine Scientific) at 1.

171. The sperm were then placed in either 1 ml Nunc cryotubes or into 0.25 ml straws and "subsequently frozen in a programmable rate freezer" using liquid nitrogen vapor. Ex. 1007 (Fugger) at 2368.

172. Fugger reports that sperm sorted for the X chromosome were used in intra-cytoplasmic sperm injection and intrauterine injection in human patients after being thawed at room temperature, centrifuged, and resuspended. Ex. 1007 (Fugger) at 2368. A total of 29 pregnancies were achieved for a 21.2% clinical pregnancy rate. *Id.* at 2368. Additionally, sperm sorted for the X chromosome were used for intrauterine insemination in 92 patients, with a pregnancy rate of 10.6%. *Id.* Of those pregnancies where the sex of the fetus was known, 88.2% were female. *Id.* Fugger specifically notes that "[p]regnancies have also been obtained with the use of frozen sorted sperm cells." *Id.* at 2369. Fugger characterizes the results as "the achievement of a substantial number of

pregnancies and deliveries of normal babies" and notes that "most of these pregnancies were achieved after simple IUI." *Id.* at 2368.

C. Claims 1-4, 8-20, 22 and 26-28 Are Unpatentable In View Of Seidel 1997 In Combination With Salisbury And Spaulding

a. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1

173. Seidel 1997 in combination with Salisbury and Spaulding renders obvious a method of freezing sex-selected sperm cells.

174. Seidel 1997 discloses the step of obtaining sperm from a male of a species of non-human mammal. Specifically, Seidel 1997 discloses obtaining sperm from bulls using an artificial vagina. *See, supra*, at ¶ 127.

175. Seidel 1997 discloses sorting of sperm cells based upon sex-type to provide a collection of sex-selected sperm cells obtained using flow cytometry or fluorescence-activated cell-sorting.

176. Seidel 1997 discloses the use of flow cytometry to separate X chromosome containing sperm from Y chromosome containing sperm. *See, supra*, at ¶¶ 131-134. Seidel 1997 discloses that separation of sperm using flow cytometry became possible "as a result of advances and discoveries involving the differential dye absorption of X- and Y-chromosome bearing sperm." *See, supra*, at ¶ 131. Seidel 1997 discloses separating the sperm into a discrete population based upon the property used to differentiate the sperm. Specifically, Seidel 1997

discloses that "the differing length of the X-chromosome and the Y-chromosome causes different levels of staining[.] Thus, by sensing the degree of dye present in the sperm cells it is possible to discriminate between X-bearing sperm and Y-bearing sperm by their differing emission levels." *See, supra*, at ¶ 133. Seidel 1997 also discloses that "by sensing some property of the cells or other items the flow cytometer can discriminate between cells based on a particular characteristic and place them in the appropriate collector." *See, supra*, at ¶ 134. Indeed, the '265 patent recognizes that the prior art taught flow cytometry and fluorescence activated cell sorting as a way of sorting sperm cells:

Flow cytometry is a preferred method for separating cells from mixed populations based on differential staining with fluorescent dyes or binding to fluorescently labeled molecules, such as antibodies or nucleic acids. In fluorescence activated cell sorting ("FACS"), cells are "sorted" into different populations based on the fluorescence intensity upon irradiation. FACS can be used for sex-selection of sperm because the X chromosome contains slightly more DNA than the Y chromosome. When sperm are stained with a fluorescent DNA-binding dye, X-chromosome bearing sperm absorb more dye than Y chromosome bearing sperm and the two populations can therefore can [sic] be separated by FACS. This strategy was discussed in U.S. Pat. No. 4,362,246 and significantly expanded upon in U.S. Pat. No. 5,135,759 (issued to

Johnson). Separation has been enhanced through the use of high speed flow cytometers, such as the MoFlo® flow cytometer produced by Cytomation, Inc. (Ft. Collins, Colo.) and described in U.S. Pat. Nos. 5,150,313, 5,602,039, 5,602,349, and 5,643,796, as well as in PCT Publication No. WO 96/12171.

Ex. 1001, 4:40-59. Thus, Seidel 1997 discloses sorting of sperm cells based upon sex-type to provide a collection of sex-selected sperm cells obtained using flow cytometry or fluorescence-activated cell-sorting.

177. As to the requirement of sorting "without the presence of protective compounds in seminal plasma," Spaulding describes in Example 1 the "sorting of sperm by flow cytometry" using fluorescent dye to distinguish and sort the X-sperm and Y-sperm. Ex. 1031 (Spaulding), 7:43-10:8. Example 1 describes the "preparation of the sperm" prior to sorting. *Id.*, 9:15-36. As part of the preparation, the sperm was washed three times and then centrifuged to remove the seminal plasma. *Id.*, 9:25-27 ("We centrifuged the cells at 483Xg for 20 minutes and resuspended them **to remove seminal plasma proteins.**" (emphasis added)). This preparation process would have removed all, or nearly all, of the seminal plasma prior to sorting.

178. A person of ordinary skill in the art would have looked to Spaulding, because it, like Seidel 1997, is directed to sorting sperm using flow

cytometry. In addition, the removal of seminal plasma through centrifugation was taught by both Spaulding and Seidel 1997. Spaulding did so prior to sorting by flow cytometry, as discussed above. And Seidel describes a centrifuging step after sorting, at conditions that would also remove the seminal plasma. Ex. 1005 (Seidel 1997), 15:32-33 ("Collected [sorted] sperm were centrifuged at 600xg for 10 min. and resuspended to 1.63×10^6 live sperm/ ml in CUE."). Whether to remove the seminal plasma before or after sorting is a matter of process design choice and an obvious variant. Furthermore, Spaulding provides a motivation to remove the seminal plasma prior to sorting. Specifically, Spaulding states that one of the objectives of the pre-sort preparation of the sperm, which included the removal of seminal plasma, was "to match the refractive indices between the sheath and sample fluid." Ex. 1031 (Spaulding), 9:35-36. For these reasons, it would have been obvious to one skilled in the art to sort sperm cells without the presence of protective compounds in seminal plasma, using flow cytometry.

179. Seidel 1997 discloses the step of cooling the sex-selected sperm. Specifically, Seidel 1997 discloses cooling the sperm to slow the sperm's metabolism and to permit its use over longer periods of time. *See, supra*, at ¶ 140.

180. Seidel 1997 discloses that the sorted sperm are suspended in an extender, after which the sperm is at a concentration of 3 to 5 million per milliliter. *See, supra*, at ¶ 139.

181. Seidel 1997 in combination with Salisbury renders obvious the next step of freezing the selected sperm sample. Specifically, Seidel 1997 notes that the separated "sample may then be cooled to slow the sperm's metabolism and to permit use over longer periods of time." *See, supra*, at ¶ 140. A person of ordinary skill in the art would have been motivated to freeze the separated sperm cells because of the numerous benefits inherent in freezing sperm, such as the ability to ship sperm long distances and the knowledge that freezing was the preferred method of preserving sperm. *See, supra*, at ¶ 70. Such a person would have been well aware of the standard methods of freezing sperm. For example, Salisbury (an earlier edition of which is cited in Seidel 1997 at page 7 of the references) discloses methods for freezing sperm cells. *See, supra*, at ¶¶ 145-157. Such a person also would have expected that separated sperm could be successfully frozen. Individuals of ordinary skill in the art had been freezing sperm from non-human mammals since at least the 1950's. *See, supra*, at ¶¶ 30-55. Furthermore, individuals of ordinary skill in the art had reported successfully freezing sperm that had been subjected to separation procedures. *See, supra*, at ¶¶ 56-72. Based on the teachings in the literature, *see, supra*, at *id.*, a person of ordinary skill in the art would have expected separated, frozen sperm to be viable after thawing.

182. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1 of the '265 patent.

b. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 2

183. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1. *See, supra*, at ¶¶ 173-182.

184. Seidel 1997 also discloses that the sperm cells are bovine or equine. Specifically, Seidel 1997 collected sperm cells from bulls. *See, supra*, at ¶ 127.

185. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 2 of the '265 patent.

c. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 3

186. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 2. *See, supra*, at ¶¶ 173-185.

187. Seidel 1997 also discloses that the insemination sample has between three hundred thousand and three million sex-selected sperm cells. Specifically, Seidel 1997 discloses that separated sperm are extended to a concentration of 3-5 million per ml. *See, supra*, at ¶ 139. A person of ordinary skill in the art would understand that 0.25 ml straws are commonly used, resulting in a sample of 1.25 million sperm at the higher concentration. Although Seidel

1997 notes that the sperm may be further diluted to the three hundred thousand range, nothing in Seidel 1997 requires such a further dilution step. And, Salisbury similarly discloses that a wide variety of amounts of bovine sperm can be successfully utilized to inseminate female cows, including amounts in the 300 thousand to 3 million range. *See, supra*, at ¶¶ 157-158.

188. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 3 of the '265 patent.

d. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 4

189. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding discloses or renders obvious all of the limitations of claims 1 and 2. *See, supra*, at ¶¶ 173-185.

190. Seidel 1997 also discloses that the insemination sample has no more than one million sex-selected sperm cells. Specifically, Seidel 1997 discloses that separated sperm are extended to a concentration of 3-5 million per ml. *See, supra*, at ¶ 139. A person of ordinary skill in the art would understand that 0.25 ml straws are commonly used, resulting in a sample of 1.25 million sperm at the higher concentration. Seidel 1997 notes that the sperm may be further diluted to a sample of no more than one million sex-selected sperm cells. *Id.*

191. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 4 of the '265 patent.

e. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 8

192. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious of claim 1. *See, supra*, at ¶¶ 173-182.

193. Seidel 1997 also discloses that the step of cooling the sperm cells comprises the step of reducing the temperature to about 5° C. Specifically, Seidel 1997 discloses that the sperm is reduced in temperature to 5° C over 75 minutes. *See, supra*, at ¶ 140.

194. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious all of the limitations of claim 8 of the '265 patent.

f. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 9

195. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 8. *See, supra*, at ¶¶ 173-182, 192-194.

196. Seidel 1997 discloses the step of reducing the temperature for a period of about 60 minutes to about 240 minutes. Seidel 1997 discloses the step of cooling the sperm. Specifically, Seidel 1997 discloses cooling the sperm to slow the sperm's metabolism and to permit its use over longer periods of time. *See, supra*, at ¶ 140. Specifically, Seidel 1997 discloses that the sperm is reduced in temperature to 5° C over 75 minutes. Ex. 1005 (Seidel 1997), 15:37-39.

197. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious of claim 9 of the '265 patent.

g. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 10

198. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1. *See, supra*, at ¶¶ 173-182.

199. Seidel 1997 also discloses a final extender further comprising a component which maintains osmolality and buffers pH. Specifically, Seidel 1997 discloses an extender containing sodium citrate. *See, supra*, at ¶¶ 136-137.

Sodium citrate was well known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality.

Ex. 1001, 6:22-37.

200. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 10 of the '265 patent.

h. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 11

201. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 10. *See, supra*, at ¶¶ 173-182, 198-200.

202. Seidel 1997 also discloses a final extender wherein the component which maintains osmolality and buffers pH is selected from the group consisting of a buffer comprising a salt, a buffer containing a carbohydrate, and any combination thereof. Specifically, Seidel 1997 discloses an extender containing sodium citrate. *See, supra*, at ¶¶ 136-137. Sodium citrate was well known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001, 6:22-37. A person of ordinary skill in the art would have known that sodium citrate is a salt.

203. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious all of the limitations of claim 11 of the '265 patent.

i. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 12

204. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 10. *See, supra*, at ¶¶ 173-182, 198-200.

205. Seidel 1997 also discloses that the component in the final extender which maintains osmolality can be selected from the group consisting of sodium citrate, Tris[hydroxymethyl]aminomethane, 200 mM Tris [hydroxymethyl]aminomethane, 175 mM to 225 mM Tris[hydroxymethyl]aminomethane, 200 mM

Tris[hydroxymethyl]aminomethane/65 mM citric acid monohydrate, 175 mM to 225 mM Tris[hydroxymethyl]aminomethane/50 mM to 70 mM citric acid monohydrate, N-Tris [hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/65 mM citric acid monohydrate, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/50 mM to 70 mM citric acid monohydrate, monosodium glutamate, milk, HEPES buffered medium, and any combination thereof. Specifically, Seidel 1997 discloses an extender containing sodium citrate. *See, supra*, at ¶¶ 136-137. Sodium citrate was well known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001, 6:22-37.

206. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding discloses or renders obvious all of the limitations of claim 12 of the '265 patent.

j. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 13

207. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11 and 12. *See, supra*, at ¶¶ 173-182, 198-206.

208. Seidel 1997 also discloses an extender with a pH in the range of about 6.5 to about 7.5. Specifically, Seidel 1997 discloses an extender containing sodium citrate. *See, supra*, at ¶¶ 136-137. Sodium citrate was well known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Salisbury reports that the optimal pH for a seminal extender is in the range of 6.5 to 7.5, depending upon the ions in the extender. Ex. 1006 (Salisbury) at 502. In particular, the optimal pH for citrate extender is 6.5 to 7.0. *Id.* Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001, 6:22-36.

209. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 13 of the '265 patent.

k. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 14

210. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 10, 11, 12 and 13. *See, supra*, at ¶¶ 173-182, 198-209.

211. Seidel 1997 in combination with Salisbury renders obvious the use of an extender comprising a cold shock protectant. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Seidel 1997 discloses that sperm should be extended with an extender and notes that other extenders can be used. *See, supra*, at ¶¶ 136-137. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 148. A person of ordinary skill would have been motivated to use these ingredients when freezing separated sperm.

212. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 14 of the '265 patent.

I. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 15

213. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11, 12, 13 and 14. *See, supra*, at ¶¶ 173-182, 198-212.

214. Seidel 1997 also discloses that the cold shock treatment is selected from the group consisting of egg yolk, 20% egg yolk, 15% to 25% egg

yolk, an egg yolk extract, milk, a milk extract, casein, albumin, lecithin, and any combination thereof. Specifically, Seidel 1997 discloses the use of egg yolk in the final extender. *See, supra*, at ¶¶ 136-137.

215. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 15 of the '265 patent.

m. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 16

216. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11, 12, 13 and 14. *See, supra*, at ¶¶ 173-182, 198-212.

217. Seidel 1997 in combination with Salisbury renders obvious the use of an extender comprising an energy source. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Seidel 1997 discloses that sperm should be extended with an extender and notes that other extenders can be used. *See, supra*, at ¶¶ 136-137. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant.

See, supra, at ¶ 148. A person of ordinary skill would have been motivated to use these ingredients when freezing separated sperm.

218. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 16 of the '265 patent.

n. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 17

219. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11, 12, 13, 14, and 16. *See, supra*, at ¶¶ 173-182, 198-212, 216-218.

220. Seidel 1997 in combination with Salisbury renders obvious the limitation that the energy source is selected from the group consisting of a saccharide, a glucose, a fructose, a 56 mM fructose, a 45 mM to 60 mM fructose, a mannose, and any combination thereof. Specifically, Seidel 1997 teaches that glucose can be used in the final extender. *See, supra*, at ¶ 137. Salisbury likewise teaches that simple sugars such as glucose and fructose can be used for an energy source. *See, supra*, at ¶ 153. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181.

221. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 17 of the '265 patent.

o. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 18

222. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11, 12, 13, 14 and 16. *See, supra*, at ¶¶ 173-182, 198-212, 216-218.

223. Seidel 1997 in combination with Salisbury renders obvious the use of a final extender comprising an antibiotic. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Seidel 1997 discloses that sperm should be extended with an extender and notes that other extenders can be used. *See, supra*, at ¶¶ 136-137. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 148. A person of ordinary skill would have been motivated to use these ingredients when freezing separated sperm.

224. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 18 of the '265 patent.

p. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 19

225. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10, 11, 12, 13, 14, 16 and 18. *See, supra*, at ¶¶ 173-182, 198-212, 216-218, 222-224.

226. Seidel 1997 in combination with Salisbury also renders obvious the use of an extender with an antibiotic selected from the group consisting of tylosin, gentamicin, lincomycin, linco-spectin, spectinomycin, penicillin, streptomycin, and any combination thereof. Seidel 1997 discloses that the extender may contain antibiotics, such as tylosin, gentamicin, and linco-spectin. *See, supra*, at ¶¶ 128, 138. Salisbury discloses that antibiotics should be included in extenders used to freeze semen and specifically teaches the use of antibiotics such as penicillin and streptomycin. *See, supra*, at ¶ 154. A person of ordinary skill in the art would have been motivated to add such an antibiotic to the final extender disclosed in Seidel 1997, because such antibiotics were commonly available to one of ordinary skill in the art, because the use of antibiotics was mandated by CSS and other rules, and because it was known that antibiotics were needed to inhibit the growth of microorganisms. *See, supra*, at ¶¶ 46-47. Indeed, as the '265 patent recognizes, "substantial bacterial growth can threaten sperm viability and increase the risk of infection of the host in artificial insemination or in vitro fertilization procedures." Ex. 1001, 7:19-22.

227. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 19 of the '265 patent.

q. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 20

228. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1 and 10. *See, supra*, at ¶¶ 173-182, 198-200.

229. Seidel 1997 in combination with Salisbury renders obvious the use of an extender comprising a cryoprotectant. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Seidel 1997 discloses that sperm should be extended with an extender and notes that other extenders can be used. *See, supra*, at ¶¶ 136-137. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 148. A person of ordinary skill would have been motivated to use these ingredients when freezing separated sperm.

230. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 20 of the '265 patent.

r. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 22

231. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claims 1, 10 and 20. *See, supra*, at ¶¶ 173-182, 198-200, 228-230.

232. Seidel 1997 in combination with Salisbury also discloses that the cryoprotectant is selected from the group consisting of glycerol, 6% glycerol, between 5% to 7% glycerol, dimethyl sulfoxide, ethylene glycol, propylene glycol, and any combination thereof. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Salisbury discloses the use of glycerol in the final extender and notes that, since the discovery of the benefits of glycerol, freezing sperm has been the preferred way of preserving sperm. *See, supra*, at ¶¶ 146, 152. A person of ordinary skill would therefore have utilized glycerol when freezing separated sperm.

233. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 22 of the '265 patent.

**s. Seidel 1997 in combination with Salisbury and
Spaulding renders obvious claim 26**

234. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1. *See, supra*, at ¶¶ 173-182.

235. Seidel 1997 in combination with Salisbury also renders obvious the step of equilibrating the discrete population of sperm cells in a final extender for a duration of about 1 hour to about 18 hours. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Such a person would have been well aware of Salisbury, which notes that fertility trials "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period of several hours" and recommends equilibrating in a final extender containing glycerol for between 4 and 18 hours. *See, supra*, at ¶¶ 152, 156. This range overlaps with the range identified in claim 26.

236. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 26 of the '265 patent.

t. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 27

237. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding obvious claims 1, 10, 20 and 22. *See, supra*, at ¶¶ 173-182, 198-200, 228-233.

238. Seidel 1997 in combination with Salisbury also renders obvious the step of equilibrating the discrete population of sperm cells in a final extender for a duration of a period not greater than 6 hours. As discussed above, a person of ordinary skill in the art would have been motivated to combine Seidel 1997's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 70, 181. Such a person would have been well aware of Salisbury, which notes that fertility trials "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period of several hours" and recommends equilibrating in a final extender containing glycerol for between 4 and 18 hours. *See, supra*, at ¶¶ 152, 156. This range overlaps with the range identified in claim 27.

239. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 27 of the '265 patent.

u. Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 28

240. As discussed above, Seidel 1997 in combination with Salisbury and Spaulding renders obvious claim 1. *See, supra*, at ¶¶ 173-182.

241. Seidel 1997 also discloses that the insemination sample has between three hundred thousand and five million sex-selected sperm cells. Specifically, Seidel 1997 discloses that separated sperm are extended to a concentration of 3-5 million per ml. *See, supra*, at ¶ 139. A person of ordinary skill in the art would understand that 0.25 ml straws are commonly used, resulting in a sample of 1.25 million sperm at the higher concentration. Although Seidel 1997 notes that the sperm may be further diluted, nothing in Seidel 1997 requires such a further dilution step. Salisbury similarly discloses that a wide variety of amounts of bovine sperm can be successfully utilized to inseminate female cows, including amounts in the 300 thousand to 5 million range. *See, supra*, at ¶¶ 157-158.

242. Thus, in my opinion, Seidel 1997 in combination with Salisbury and Spaulding renders obvious all of the limitations of claim 28 of the '265 patent.

D. Claims 1-4, 8-20, 22 and 26-28 Are Unpatentable In View Of Fugger And Salisbury

a. Fugger in combination with Salisbury renders obvious claim 1

243. Fugger discloses a method of freezing sex-selected sperm cells.

See, supra, at ¶¶ 163-171.

244. Fugger renders obvious the step of obtaining sperm from a male of a species of non-human mammal. Fugger discloses the step of obtaining sperm from a male of the human species. *See, supra*, at ¶¶ 163-164. However, a person of ordinary skill in the art would know how to obtain sperm from the male of a species of non-human mammal. A person of ordinary skill in the art would also know that the separation method described in Fugger would be applicable to non-human mammalian sperm. Numerous references taught both how to collect sperm from non-human animals and that flow cytometry could be used to separate the sperm of non-human mammals. *See, supra*, at, e.g., ¶¶ 22-26, 58, 78-79.

Individuals had been using flow cytometry to sort sperm from non-human mammals since at least 1992. *See, supra*, at ¶¶ 24-26. Indeed, Fugger itself states that the research it reports was undertaken "[b]ased on the birth of over 400 normal offspring from four species of animals." *See, supra*, at ¶ 163. And the benefits of frozen sperm from species such as bovine were well known. *See, supra*, at ¶ 70. Thus, after reading Fugger, a person of ordinary skill in the art would have been

motivated to separate sperm from non-human mammals and thereafter freeze that sperm. Furthermore, such a person would have expected to be successful.

Individuals of ordinary skill in the art had been freezing sperm from non-human mammals since at least the 1950's. *See, supra*, at ¶¶ 30-37. Furthermore, individuals of ordinary skill in the art had reported successfully freezing sperm that had been subjected to separation procedures. *See, supra*, at ¶¶ 56-72. Thus, a person of ordinary skill in the art would have expected, after reading Fugger, that sperm from non-human animals could have been separated and frozen and thereafter successfully used in artificial insemination.

245. Fugger discloses sorting sperm cells based upon sex-type.

Fugger states that the "specimens were sorted by FCS [*i.e.*, flow cytometry separation] as previously described (Johnson et al., 1993)." In particular, Fugger discloses differentiating X chromosome bearing sperm cells and Y chromosome bearing sperm cells based on how those cells fluoresce in a flow cytometer, which is based on the amount of DNA contained within the cells. *See, supra*, at ¶¶ 167-168. Fugger discloses separating the sperm into a discrete population based upon the property used to differentiate the sperm. Specifically, Fugger discloses separating X chromosome bearing sperm and Y chromosome bearing sperm cells using flow cytometry, which is based on the amount of DNA contained within the cells. *See, supra*, at ¶¶ 167-169. A person of ordinary skill in the art would

understand that X bearing chromosomes fluoresce brighter due to the fact that they contain more DNA than Y bearing chromosomes. *See, supra*, at ¶ 26. Fugger also discloses the step of staining the sperm cells with a fluorochrome which binds to DNA within the sperm cells. More specifically, Fugger discloses staining the sperm cells with Hoechst 33342, a fluorochrome bisbenzimidazole, which binds to DNA within the sperm cells. *See, supra*, at ¶ 166. Fugger also discloses that the step of assessing the amount of DNA within each sperm cell comprises the steps of (a) exciting the fluorochrome which binds to DNA with the sperm cells, and (b) analyzing light emitted by the fluorochrome bound to DNA within the sperm cells. Specifically, Fugger discloses that "[f]luorescence emitted from each sperm was detected." *See, supra*, at ¶ 168. A person of ordinary skill in the art would understand that Fugger first excited the fluorochrome bound to DNA and then analyzed it. *See, supra*, at ¶ 26.

246. Fugger reports that the sorted sperm was "collected." *See, supra*, at ¶ 169.

247. Fugger also discloses the sorting of the semen was done without the presence of protective compounds in seminal plasma. Fugger states that the "[s]pecimens to be sorted were extended, centrifuged, resuspended [and] filtered" as part of the sperm preparation and prior to the flow cytometry separation. Ex. 1007 (Fugger) at 2367. The conditions specified by Fugger would

have removed all, or nearly all, of the seminal plasma prior to the sorting. *See, supra*, at ¶¶ 28, 157, 165. For the specific pre-sort conditions, Fugger cites to an earlier paper, Johann 1989. Ex. 1007 (Fugger) at 2367. Johann 1989 describes the sequential centrifugation, removal of supernatant and resuspension of the sperm, three times, and specifies the centrifugation conditions of each (750 x g for 15 minutes, 200 x g for 5 minutes and 200 x g for 5 minutes). Ex. 1032 (Johann 1989) at 686. Those conditions would have removed the seminal plasma.

248. Fugger in combination with Salisbury also renders obvious the step of cooling the sperm cells to 5° C. Fugger describes freezing separated human sperm. One of ordinary skill in the art would have understood that slightly different methods may be required when freezing sperm of other species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches the importance of cooling bovine sperm prior to freezing and notes that sperm can be cooled to 5° C. *See, supra*, at ¶¶ 155-156.

249. Fugger also discloses suspending sex-selected sperm cells in and extender prior to freezing. In particular, Fugger described that: "Sorted specimens to be frozen were extended with TEST yolk buffer freezing medium" and then subsequently frozen. Ex. 1007 (Fugger) at 2368. Fugger does not specifically mention that the concentration of the sperm in the extender is about 5

million per milliliter to about 10 million per milliliter. However, one of ordinary skill in the art would have understood that slightly different methods may be required when freezing sperm of other species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches that sperm concentrations of 5-10 million per milliliter had been frozen and subsequently used for successful inseminations. Ex. 1006 (Salisbury) at 517-518. *See, supra*, at ¶¶ 157-158. In particular, Salisbury reports data from a study indicating that successful pregnancies were obtained after insemination with $.375 \times 10^6$, $.75 \times 10^6$ and 3×10^6 live sperm after thawing. Ex. 1006 (Salisbury) at 518, citing Ex. 1034 (Jondet 1972). Based on the underlying data, pre-freezing concentrations of 7.8 million sperm per milliliter and 5.6 million sperm per milliliter were among those used. *Id.*

250. Fugger discloses freezing the sorted sperm sample.

Specifically, as part of the post-sort specimen cryopreservation, Fugger states separated sperm were placed into cryotubes or straws "and subsequently frozen in a programmable rate freezer." *See, supra*, at ¶ 171.

251. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 1 of the '265 patent.

b. Fugger in combination with Salisbury renders obvious claim 2

252. As discussed above, Fugger renders obvious claim 1. *See, supra*, at ¶¶ 243-251.

253. Fugger also renders obvious the use of bovine or equine sperm cells. As noted above, a person of ordinary skill in the art would understand that the method described in Fugger could be utilized with bovine sperm. *See, supra*, at ¶ 244.

254. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 2 of the '265 patent.

c. Fugger in combination with Salisbury renders obvious claim 3

255. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 2. *See, supra*, at ¶¶ 243-254.

256. Fugger in combination with Salisbury also renders obvious the step of isolating between three hundred thousand and about three million sex-selected sperm. Fugger describes the separation and subsequent use of separated human sperm to achieve fertilizations. One of ordinary skill in the art would have understood that successful fertilizations in different species might require different amounts of sperm and, if attempting to inseminate a female using frozen bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the

bovine insemination industry. Salisbury discloses that a wide variety of amounts of bovine sperm can be successfully utilized to inseminate female cows, including amounts in the 300 thousand to 3 million range. *See, supra*, at ¶¶ 157-158. One of skill in the art would have known that 300 thousand to 3 million sperm could be used to inseminate cows.

257. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 3 of the '265 patent.

d. Fugger in combination with Salisbury renders obvious claim 4

258. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 2. *See, supra*, at ¶¶ 243-254.

259. Fugger in combination with Salisbury also renders obvious the step of isolating a number of bovine sperm cells of no more than about one million. Fugger describes the separation and subsequent use of separated human sperm to achieve fertilizations. One of ordinary skill in the art would have understood that successful fertilizations in different species might require different amounts of sperm and, if attempting to inseminate a female using frozen bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that a wide variety of amounts of bovine sperm can be successfully utilized to inseminate female cows, including

amounts of no more than about 1 million. *See, supra*, at ¶ 158. One of skill in the art would have known that 1 million sperm could be used to inseminate cows.

260. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 4 of the '265 patent.

e. Fugger in combination with Salisbury renders obvious claim 8

261. As discussed above, Fugger in combination with Salisbury renders obvious claim 1. *See, supra*, at ¶¶ 243-251.

262. Fugger in combination with Salisbury also renders obvious the step of cooling the sperm cells to 5° C. Fugger describes freezing separated human sperm. One of ordinary skill in the art would have understood that slightly different methods may be required when freezing sperm of other species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches the importance of cooling bovine sperm prior to freezing and notes that sperm can be cooled to 5° C. *See, supra*, at ¶¶ 154-156.

263. Thus, in my opinion, Fugger in combination with Salisbury renders obvious of claim 8 of the '265 patent.

f. Fugger in combination with Salisbury renders obvious claim 9

264. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 8. *See, supra*, at ¶¶ 243-251, 261-263.

265. Fugger in combination with Salisbury also renders obvious the step of cooling the sperm cells for a period of 60 minutes to about 240 minutes. Fugger describes freezing separated human sperm. One of ordinary skill in the art would have understood that slightly different methods may be required when freezing sperm of other species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches the importance of slow cooling bovine sperm prior to freezing. *See, supra*, at ¶¶ 154-156. A person of ordinary skill in the art, when freezing bull sperm, would have cooled it slowly to avoid "cold shock." *Id.* Salisbury reports benefits of cooling over a period of 4 hours. *Id.*

266. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 9 of the '265 patent.

g. Fugger in combination with Salisbury renders obvious claim 10

267. As discussed above, Fugger in combination with Salisbury renders obvious claim 1. *See, supra*, at ¶¶ 243-251.

268. Fugger in combination with Salisbury also discloses a final extender further comprising a component which maintains osmolality and buffers pH. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches that extenders used in freezing bovine semen typically contain a component which maintains osmolality and buffers pH. *See, supra*, at ¶¶ 148-149.

269. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 10 of the '265 patent.

h. Fugger in combination with Salisbury renders obvious claim 11

270. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 10. *See, supra*, at ¶¶ 243-251, 267-269.

271. Fugger in combination with Salisbury also discloses a final extender wherein the component which maintains osmolality and buffers pH is selected from the group consisting of a buffer comprising a salt, a buffer containing a carbohydrate, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if

attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Specifically, Salisbury discloses an extender containing sodium citrate. *See, supra*, at ¶ 149. Sodium citrate was well known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001 ('265 patent), 6:22-36. A person of ordinary skill in the art would have incorporated sodium citrate when freezing bull sperm and would have known that sodium citrate is a salt.

272. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 11 of the '265 patent.

i. Fugger in combination with Salisbury renders obvious claim 12

273. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 10. *See, supra*, at ¶¶ 243-254, 267-269.

274. Fugger in combination with Salisbury also discloses that the component in the final extender which maintains osmolality can be selected from the group consisting of sodium citrate, Tris[hydroxymethyl]aminomethane, 200 mM Tris [hydroxymethyl]aminomethane, 175 mM to 225 mM Tris[hydroxymethyl]aminomethane, 200 mM Tris[hydroxymethyl]aminomethane/65 mM citric acid monohydrate, 175 mM to

225 mM Tris[hydroxymethyl]aminomethane/50 mM to 70 mM citric acid monohydrate, N-Tris [hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/65 mM citric acid monohydrate, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/50 mM to 70 mM citric acid monohydrate, monosodium glutamate, milk, HEPES buffered medium, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches that sodium citrate can be used as a buffer to maintain osmolality and buffers pH. *See, supra*, at ¶ 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001 ('265 patent), 6:22-36.

275. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 12 of the '265 patent.

j. Fugger in combination with Salisbury renders obvious claim 13

276. As discussed above, Fugger in combination with Salisbury renders obvious claims 1, 10, 11 and 12. *See, supra*, at ¶¶ 243-251, 267-275.

277. Fugger in combination with Salisbury also discloses an extender that has a pH in the range of about 6.5 to about 7.5. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Specifically, Salisbury discloses an extender containing sodium citrate. *See, supra*, at ¶ 149. Sodium citrate was well-known in the art as a buffer that maintains osmolality and buffers pH. *See, supra*, at ¶¶ 42, 149. Indeed, the '265 patent identifies sodium citrate as a well-known example of a buffer that maintains pH and osmolality. Ex. 1001 ('265 patent), 6:22-37. Salisbury reports that the optimal pH for a seminal extender is in the range of 6.5 to 7.5, depending upon the ions in the extender. Ex. 1006 (Salisbury) at 502. In particular, the optimal pH for Tris and citrate extender is 6.5 to 7.0. *Id.*

278. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 13 of the '265 patent.

k. Fugger in combination with Salisbury renders obvious claim 14

279. As discussed above, Fugger in combination with Salisbury renders obvious all of the limitations of claims 1, 10, 11, 12 and 13. *See, supra*, at ¶¶ 243-251, 267-278.

280. Fugger in combination with Salisbury renders obvious the use of an extender comprising a cold shock protectant. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 149. A person of ordinary skill in the art, when freezing bull sperm, would have utilized a final extender containing these ingredients.

281. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 14 of the '265 patent.

I. Fugger in combination with Salisbury renders obvious claim 15

282. As discussed above, Fugger in combination with Salisbury renders obvious claims 1, 10, 11, 12, 13 and 14. *See, supra*, at ¶¶ 243-251, 267-281.

283. Fugger in combination with Salisbury also discloses that the cold shock treatment is selected from the group consisting of egg yolk, 20% egg yolk, 15% to 25% egg yolk, an egg yolk extract, milk, a milk extract, casein, albumin, lecithin, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches that egg yolk is used extensively in extenders and that it protects against cold shock. *See, supra*, at ¶¶ 148, 151. A person of ordinary skill in the art would have known to use egg yolk in the final extender when freezing bull sperm.

284. This, in my opinion, Fugger in combination with Salisbury renders obvious claim 15 of the '265 patent.

m. Fugger in combination with Salisbury renders obvious claim 16

285. As discussed above, Fugger renders obvious claims 1, 10, 11, 12, 13 and 14. *See, supra*, at ¶¶ 243-251, 267-281.

286. Fugger in combination with Salisbury renders obvious the use of an extender comprising an energy source. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that the basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 148. A person of ordinary skill in the art, when freezing bull sperm, would have utilized a final extender containing these ingredients.

287. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 16 of the '265 patent.

n. Fugger in combination with Salisbury renders obvious claim 17

288. As discussed above, Fugger renders obvious claims 1, 10, 11, 12, 13, 14 and 16. *See, supra*, at ¶¶ 243-251, 267-281, 285-287.

289. Fugger in combination with Salisbury renders obvious the limitation that the energy source is selected from the group consisting of a saccharide, a glucose, a fructose, a 56 mM fructose, a 45 mM to 60 mM fructose, a mannose, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury teaches that simple sugars such as glucose and fructose can be used for an energy source. *See, supra*, at ¶ 153. A person of ordinary skill in the art, when freezing bull sperm, would have utilized a simple sugar such as glucose or fructose.

290. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 17 of the '265 patent.

o. Fugger in combination with Salisbury renders obvious claim 18

291. As discussed above, Fugger discloses or renders obvious all of the limitations of claims 1, 10, 11, 12, 13, 14 and 16. *See, supra*, at ¶¶ 243-251, 267-281, 285-287.

292. Fugger in combination with Salisbury also renders obvious the use of an extender with an antibiotic. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different

extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that antibiotics should be included in extenders used to freeze semen and specifically teaches the use of antibiotics. *See, supra*, at ¶ 154. Indeed, as the '265 patent recognizes, "substantial bacterial growth can threaten sperm viability and increase the risk of infection of the host in artificial insemination or in vitro fertilization procedures." Ex. 1001 ('265 patent), 7:19-22. A person of ordinary skill in the art, when freezing bull sperm, would have used an antibiotic.

293. Thus, in my opinion, Fugger 1989 in combination with Salisbury renders obvious claim 18 of the '265 patent.

p. Fugger in combination with Salisbury renders obvious claim 19

294. As discussed above, Fugger in combination with Salisbury renders obvious claims 1, 10, 11, 12, 13, 14, 16 and 18. *See, supra*, at ¶¶ 243-251, 267-281, 285-287, 291-293.

295. Fugger in combination with Salisbury also renders obvious the use of an extender with an antibiotic selected from the group consisting of tylosin, gentamicin, lincomycin, linco-spectin, spectinomycin, penicillin, streptomycin, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different

extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that antibiotics should be included in extenders used to freeze semen and specifically teaches the use of antibiotics such as penicillin and streptomycin. *See, supra*, at ¶ 154. Indeed, as the '265 patent recognizes, "substantial bacterial growth can threaten sperm viability and increase the risk of infection of the host in artificial insemination or in vitro fertilization procedures." Ex. 1001 ('265 patent), 7:19-22. A person of ordinary skill in the art, when freezing bull sperm, would have used an antibiotic.

296. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 19 of the '265 patent.

q. Fugger in combination with Salisbury renders obvious claim 20

297. As discussed above, Fugger in combination with Salisbury renders obvious claims 1 and 10. *See, supra*, at ¶¶ 243-251, 267-269.

298. Fugger in combination with Salisbury renders obvious the use of an extender comprising a cryoprotectant. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that the

basic components of extenders used when freezing sperm include organic materials to protect against cold shock, an energy source, an antibiotic and a cryoprotectant. *See, supra*, at ¶ 148. A person of ordinary skill in the art, when freezing bull sperm, would have utilized a final extender containing these ingredients.

299. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 20 of the '265 patent.

r. Fugger in combination with Salisbury renders obvious claim 22

300. As discussed above, Fugger in combination with Salisbury renders obvious claims 1, 10 and 20. *See, supra*, at ¶¶ 243-251, 267-269, 297-299.

301. Fugger in combination with Salisbury also discloses that the cryoprotectant is selected from the group consisting of glycerol, 6% glycerol, between 5% to 7% glycerol, dimethyl sulfoxide, ethylene glycol, propylene glycol, and any combination thereof. Fugger describes the use of an extender for human sperm. One of ordinary skill in the art would have understood that different extenders could be used with different species and, if attempting to freeze bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses the use of glycerol in the final extender and teaches that it was critical to freezing sperm successfully. *See, supra*, at ¶¶ 146, 152. A person of ordinary skill in the art, when freezing bull sperm, would have utilized glycerol.

302. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 22 of the '265 patent.

s. Fugger in combination with Salisbury renders obvious claim 26

303. As discussed above, Fugger in combination with Salisbury renders obvious claim 1. *See, supra*, at ¶¶ 243-251.

304. Fugger in combination with Salisbury also renders obvious the step of equilibrating the discrete population of sperm cells in a final extender for a period of about 1 hour to 18 hours. As discussed above, a person of ordinary skill in the art would have been motivated to combine Fugger's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 69-72, 249. Such a person would have been well aware of Salisbury, which notes that fertility trials "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period" of several hours and recommends equilibrating for between 4 and 18 hours. *See, supra*, at ¶ 156. This range overlaps with the range identified in claim 26 of the '265 patent.

305. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 26 of the '265 patent.

t. Fugger in combination with Salisbury renders obvious claim 27

306. As discussed above, Fugger in combination with Salisbury discloses or renders obvious all of the limitations of claims 1, 10, 20 and 22. *See, supra*, at ¶¶ 243-251, 267-269, 297-302.

307. Fugger in combination with Salisbury also renders obvious the step of equilibrating the discrete population of sperm cells in a final extender for a period of not greater than 6 hours. As discussed above, a person of ordinary skill in the art would have been motivated to combine Fugger's teaching of separated sperm with Salisbury's teaching of frozen sperm in light of the numerous benefits of frozen sperm and the recognition that freezing sperm was the preferred method of preserving sperm. *See, supra*, at ¶¶ 69-72, 249. Such a person would have been well aware of Salisbury, which notes that fertility trials "clearly established the beneficial influence on the fertility of bull spermatozoa of an equilibration period" of several hours and recommends equilibrating for between 4 and 18 hours. *See, supra*, at ¶ 156. This range overlaps with the range identified in claim 27.

308. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 27 of the '265 patent.

u. Fugger in combination with Salisbury renders obvious claim 28

309. As discussed above, Fugger in combination with Salisbury renders obvious claim 1. *See, supra*, at ¶¶ 243-251.

310. Fugger in combination with Salisbury also renders obvious an insemination sample having between three hundred thousand and about five million sex selected sperm. Fugger describes the separation and subsequent insemination with separated human sperm. One of ordinary skill in the art would have understood that successful insemination in different species might require different amounts of sperm and, if attempting to inseminate a female using frozen bovine sperm, would have turned to Salisbury as it is one of the most respected texts in the bovine insemination industry. Salisbury discloses that a wide variety of amounts of bovine sperm can be successfully utilized to inseminate female cows, including amounts in the 300 thousand to 5 million range. *See, supra*, at ¶¶ 157-158. One of skill in the art would have known that 300,000 to 5,000,000 sperm could be used to inseminate cows.

311. Thus, in my opinion, Fugger in combination with Salisbury renders obvious claim 28 of the '265 patent.

VI. CONCLUSION

312. It is my conclusion that claims 1-4, 8-20, 22, and 26-28 of the '265 patent are unpatentable.

VII. APPENDIX A

MATERIALS CONSIDERED by Dr. Marvin M. Pace

Exhibit No.	Reference Name
1001	U.S. Patent No. 7,208,265 ("265 patent")
1002	U.S. Patent No. 7,208,265 File History ("265 File History")
1004	<i>Curriculum vitae</i> of Dr. Marvin M. Pace
1005	U.S. Patent No. 7,195,920 ("Seidel 1997")
1006	Salisbury et al., Physiology of Reproduction and Artificial Insemination in Cattle, 2d ed. (1978) ("Salisbury")
1007	E.F. Fugger, S.H. Black, K. Keyvanfar and J.D. Schulman, "Births of normal daughters after MicroSort sperm separation and intrauterine insemination, in-vitro fertilization, or intracytoplasmic sperm injection," Vol. 13, No. 9 of Human Reproduction (1998) ("Fugger")
1008	U.S. Patent No. 5,135,759 ("Johnson 1992")
1009	Polge, C. and Rowson, L.E.A., "Long-term Storage of Bull Semen Frozen at Very Low Temperatures (-79° C.)," in "Report of The II. International Congress of Physiology and Pathology of Animal Reproduction and of Artificial Insemination," Vol. 111 (1952) ("Polge and Rowson 1952")

1010	LaSalle, B., "Introduction," in "The Integrity of Frozen Spermatozoa, Proceedings of a Round-Table Conference held on April 6-7, 1976," National Academy of Sciences (1978) ("LaSalle 1978")
1011	Herman, H.A. and Madden, F.W., "The Artificial Insemination of Dairy and Beef Cattle: A Handbook and Laboratory Manual" 6 th Ed. (1980) ("Herman and Madden 1980")
1012	Graham, E.F., "Fundamentals of the Preservation of Spermatozoa," in "The Integrity of Frozen Spermatozoa, Proceedings of a Round-Table Conference held on April 6-7, 1976," National Academy of Sciences (1978) ("Graham 1978")
1013	Berndtson, W.E. and Pickett, B.W, "Techniques for The Cryopreservation and Field Handling of Bovine Spermatozoa," in "The Integrity of Frozen Spermatozoa, Proceedings of a Round-Table Conference held on April 6-7, 1976," National Academy of Sciences (1978) ("Berndtson and Pickett 1978")
1014	Bulletin 621, "Preservation of Bull Semen at Sub-Zero Temperatures," University of IL Ag. Exp. Station (1957) ("Bulletin 621")
1015	Doak, "CSS Implementation of New Antibiotic Combination," Proceedings of the 11 th Tech. Conf. on Art. Insemination and Reproduction (1986) ("Doak 1986")

1016	Seidel et al., "Insemination of Heifers with Sexed Frozen or Sexed Liquid Semen," 51:1 Theriogenology: An Int'l J. of Reproduction, 400 (1999) ("Seidel 1999")
1017	Seidel, "Fertility of Bulls on the Edge of the Dose-Response Curve form Numbers of Sperm Per Inseminate," in Proceedings of the 17 th Technical Conference on Artificial Insemination and Reproduction (1998) ("Seidel 1998")
1018	U.S. Patent No. 3,854,470 issued in 1974 to Augspurger ("Augspurger 1974")
1019	U.S. Patent No. 4,092,229 (Bhattacharya 1978)
1020	U.S. Patent No. 4,083,957 (issued to Lang 1978)
1021	U.S. Patent No. 4,362,246 (issued to Adair 1982)
1022	A. A. Luderer, "Separation of Bovine Spermatozoa by Density on Water Insoluble Newtonian Gels and Their Use for Insemination," Bio. of Repro., Vol. 26, pages 813-24 (1982) ("Luderer 1982")
1023	U.S. Patent No. 4,474,875 ("Shrimpton 1984")
1024	Beal, W.E., et al., "Sex Ratio after Insemination of Bovine Spermatozoa Isolated using a Bovine Serum Albumin Gradient," 58 J. Anim. Sci. 1432-36 (1984) ("Beal 1984")

1025	Foot, "Normal Dev. Of Fetuses Resulting from Holstein Semen Processed for Sex Separation," Theriogenology, Vol. 24, No. 1, pages 197-202 (1985)
1026	U.S. Patent No. 4,007,087 (issued to Ericsson) ("Ericsson 1997")
1027	Polge, "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen IVF Procedures," in Proceedings of the 16 th Tech. Conf. on Artificial Insemination and Reproduction (1996)
1028	The American Heritage College Dictionary, 3 rd Ed. (1997)
1029	Catalog No. 90128; Irvine Scientific
1030	File History of U.S. Serial No. 09/577,246 ("246 File History")
1031	U.S. Patent No. 5,021,244 ("Spaulding")
1032	R. Johann, R.S. Jeyendranm, J.P.W. Vermedien, L. J.D. Zaneveld published "Human sperm selection by glass wool filtration and two-layer, discontinuous Percoll gradient centrifugation," in Vol. 51, No. 4 of Fertility and Sterility ("Johann 1989")
1033	U.S. Patent No. 7,820,425 ("425 Patent")

1034	Jondet, R. "Contribution to the assessment of the minimum number of frozen spermatozoa necessary to obtain fertilization in the cow," 7th Int'l Congress on Animal Reproduction and Artificial Insemination, Vol. 2 (1972) ("Jondet 1972")
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