Paper No. 1

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ABS GLOBAL, INC.,
Petitioner

v.

XY, LLC,
Patent Owner

Patent No. 9,365,822
Issued: June 14, 2016
Filed: February 11, 2013
Inventors: George E. Seidel, Lisa A. Herickhoff, and John L. Schenk

Title: SYSTEMS AND METHOD FOR SORTING CELLS

Inter Partes Review No. IPR2018-01224

PETITION FOR *INTER PARTES* REVIEW

Table of Contents

Page

I. MANDATORY NOTICES

A. Real Party-In-Interest (§ 42.8(b)(1))

The real party-in-interest is ABS Global, Inc., located at 1525 River Road, DeForest, Wisconsin 53532; and Genus plc, located at Matrix House, Basing View, Hampshire RG21 4DZ, UK.

B. Other Proceedings (§ 42.8(b)(2))

The '822 Patent is the subject of litigation in the United States District Court for the United States District Court for the District of Wisconsin (Civil Action Case No. 17-cv-466), which names ABS Global, Inc. and Genus plc, among others, as defendants. The '822 Patent is also the subject of pending litigation in the United States District Court for the District of Colorado, *XY, LLC v. Trans Ova Genetics*, LC, 1:17-cv-00944 (which was transferred from an earlier filed case in the Western District of Texas between the same parties, 6:16-cv-00447). Petitioner was served with an action for infringement on June 12, 2017.

C. Lead and Backup Lead Counsel (§ 42.8(b)(3))

Lead Counsel	Backup Lead Counsel
Jeffrey P. Kushan	Paul J. Zegger
Reg. No. 43,401	Reg. No. 33,821
jkushan@sidley.com	pzegger@sidley.com
(202) 736-8914	(202) 736-8060

D. Service on Petitioner (§ 42.8(b)(4))

Service on Petitioner may be made by e-mail (IPRNotices@sidley.com) or by mail or hand delivery to: Sidley Austin LLP, 1501 K Street, N.W.,

Washington, D.C. 20005. The fax number for Lead Counsel and Backup Counsel is (202) 736-8711.

II. INTRODUCTION

Citric acid containing solutions have been used for decades in the collection and processing of bovine sperm for artificial insemination. For example, buffered solutions termed "extenders" are added to bull semen when it is collected in order to dilute the ejaculate, which is then put into "straws," frozen and thawed, and ultimately used to inseminate cows. The added extenders protect the sperm cells from stresses associated with being removed from the seminal fluid and placed in a diluted aqueous environment, thereby yielding straws with higher numbers of viable sperm that will inseminate cows with a higher frequency.

The practice of collecting, processing and using processed bovine sperm to inseminate cows was revolutionized in the late 1980's with the development of flow cytometry systems that could separate live sperm into populations consisting (predominantly) of one sex chromosome, thereby enabling breeders to preferentially breed one sex of animal. This so-called Johnson technique (named after its developer, Lawrence Johnson) is credited by contested patent as being "so significant an advancement that it has for the first time made the commercial separation of such sperm feasible." '822 Patent (Ex. 1001), 2:6-10.

Johnson-1992 (Ex. 1005) describes and claims this groundbreaking work. It illustrates the Johnson technique by successfully sorting and using rabbit and swine sperm to impregnate females. In these examples, sperm was mixed in a pre-sort buffered media, stained and then sorted using a modified flow cytometer. The sheath fluid used in the experiments was 10 mM phosphate-buffered saline with 0.1% bovine serum albumin (BSA), and the sorted sperm were collected in tubes containing "a test yolk extender." *Id*., 6:38-40-41.

Claim 11 of the '822 Patent differs from the examples in Johnson-1992 patent in a single respect – it recites that the "sheath" and "collector" fluids "include[] a citric acid." But to an ordinary artisan, adapting the Johnson scheme to use sheath and collection fluids that include a citric acid would have been obvious well before 1997, particularly when adapting Johnson's examples to work with bovine sperm, one of the most commercially important types of sperm.

Initially, Johnson-1992 explains that its rabbit and swine examples were representative, and that "minor modifications" to them were anticipated. Johnson-1992, 4:65-5:3. Selecting different reagents commonly used in handling other species of sperm is exactly the type of "minor modification" envisioned in Johnson-1992.

Johnson-1992 also explained that an extender should be added to the collection container to offset the potential impact of dilution that occurs when the

sheath fluid collects in the collection container over time. Johnson-1992, 4:51-56; 6:40-46. An ordinary artisan would know to select an extender that was appropriate for the type of sperm being sorted, and the most widely used bovine sperm extenders included a citric acid, as Salisbury-1978 explains.

Johnson-1992 also emphasized that the fluids used throughout its flow cytometry process – including the sheath and collection fluids – should "maintain[] viability of the sperm." *Id.*, 2:50-8. An ordinary artisan would have understood this to be indicating that a buffer should be used as the sheath fluid that would not only would maintain the viability of bovine sperm, but also would be compatible with the extender used in the collection fluid. As Garcia-1989-III teaches, buffer systems based on citric acid meet both criteria – they showed equivalent or superior results for maintaining the viability of bovine sperm as buffers based on phosphates, and would be compatible with commonly used bovine sperm extenders that include a citric acid. An ordinary artisan would thus have considered a sheath fluid containing a citric acid to be a known and predictable alternative to the PBS-based sheath fluid used in the examples in Johnson-1992 when sorting bovine sperm.

Consequently, an ordinary artisan would have found it obvious to adapt the flow cytometry-based sorting methods described in Johnson-1992 to use sheath and collection fluids that include a citric acid based on Salisbury-1978 and Garcia-

1989-III when sorting bovine sperm. Because the sole distinction between claim 11 and Johnson-1992 would have been obvious to a skilled person, claim 11 should be cancelled. Petitioner respectfully requests the Board institute *inter partes* review and do so.

III. PROPOSED GROUND; CERTIFICATIONS

Ground One: Claim 11 of the '822 Patent is unpatentable under 35 U.S.C. § 103 as being obvious based on Johnson-1992 in view of Salisbury-1978 (Ex. 1006), and further in view of Garcia-1989-III (Ex. 1007).

The precise reasons why the claim is unpatentable are provided in § VI; evidence relied upon in support of the petition is listed in Attachment B.

Patent Owner may contend the Board should decline to institute trial based on 35 U.S.C. § 325(d). Petitioner believes no justification exists for doing so. For example, certain art including Garcia-1989-III, was not considered during examination, nor were the combined teachings of Johnson-1992, Salisbury-1978 and Garcia-1989-III. The Examiner also did not consider expert testimony relevant to reasons to combine those teachings and the beliefs and expectations of the ordinary artisan. The Board should therefore decline to exercise its discretion under § 325(d). *See, e.g.*, *Amneal Pharmaceuticals LLC v. Purdue Pharma L.P.*, IPR2016-01413, Paper 9 at 23 (P.T.A.B. Jan. 18, 2017) (declining to exercise discretion because the petition "…presents additional arguments and evidence

beyond what was already considered by the Examiner, including those presented in the [Expert's] Declaration").

ABS Global, Inc. ("Petitioner") certifies it is not barred or estopped from requesting *inter partes* review of U.S. Patent No. 9,365,822 ("'822 Patent") (Ex. 1001). Neither Petitioner, nor any party in privity with Petitioner, has (i) filed a civil action challenging the validity of any claim of the '822 Patent; or (ii) been served a complaint alleging infringement of the '822 Patent more than a year prior to the present date. Also, the '822 Patent has not been the subject of a prior *inter partes* review or a finally concluded district court litigation involving Petitioner.

The Director is authorized to charge the fee specified by 37 CFR § 42.15(a) to Deposit Account No. 50-1597.

IV. KNOWLEDGE OF THE ORDINARY ARTISAN BEFORE 1997

At least one year before December 31, 1997 – the earliest filing date claimed by the '822 Patent (before 1997), an ordinary artisan would have had extensive knowledge about how flow cytometry works, how it had been adapted to sort sperm, and how different types of sperm should be handled to preserve their viability for use in artificial insemination. Pace-Decl. ¶ 17.

A. Flow Cytometry to Sort Sperm Was Well Established Before 1997

1. Basic Flow Cytometry Principles

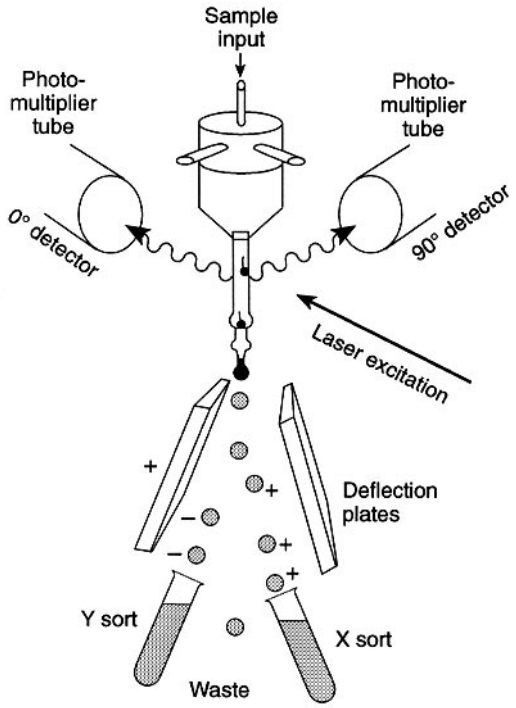
As the '822 Patent acknowledges, "[f]low cytometry in general is a technique which is well understood" and "those skilled in the art can easily

understand the basic principles involved." '822 Patent, 5:40-7. A flow cytometer is an apparatus that measures properties of individual cells in solution. Shapiro-1995 (Ex. 1008). It operates by passing cells within a fluid stream single file past a detector that measures properties of the cells by interrogating them with a light source, typically a laser. To facilitate measurement, cells being evaluated are labeled with a fluorescent agent prior to introduction into the flow cytometer. Shapiro-1995, 3. Fluid containing a sample of labeled cells ("sample" or "pre-sort" fluid) is then introduced into the flow cytometer, and additional fluid ("sheath fluid") is introduced to propel the cells through the apparatus past the detector. Shapiro-1995, 15.

2. Johnson Innovated Flow Cytometry to Enable Isolation of Viable Sexed Sperm

In the late 1980's, flow cytometers were adapted to enable the separation of sperm based on their sex characteristic for use in artificial insemination. Pace-Decl. ¶¶ 46-49 (Ex. 1003); Johnson-1989 (Ex. 1009); Johnson-1991 (Ex. 1010). The adaptations included, *inter alia*, a sorting mechanism that could divert individual sperm cells based on their detected sex characteristic into one or more collection containers, thereby yielding samples of sperm consisting of predominantly one type of sperm (i.e., having either an X- or a Y-chromosome).

The following figure illustrates a modified flow cytometer used for sorting cells from a Johnson paper before 1997:



Cran-1996 (Ex. 1011), 357, Figure 1a.

Distinguishing sperm by sex characteristic is possible because an X-chromosome contains slightly more DNA than the Y-chromosome. Pace-Decl. ¶¶ 50-51; Johnson-1992, 1:62-4 ("[t]he difference in total DNA between X-bearing sperm and Y-bearing sperm is 3.4% in boar, 3.8% in bull, and 4.2% in ram sperm."). The greater amount of DNA in X-chromosomes causes them to absorb more of a DNA intercalating fluorescent dye (*e.g.*, a Hoechst dye) and to thereby emit more fluorescent light when interrogated by the detection unit of a flow cytometer. Johnson-1992, 3:4-13.

3. The Johnson Technique Was Widely Followed, Including by the '822 Patent

Procedures for sex sorting sperm using flow cytometry were well-established before 1997. Notably, the '822 Patent credits Johnson's work in the late 1980s and early 1990s for "significantly" expanding earlier attempts to sex-sort sperm, referring to the method described in Johnson-1992 as the "Johnson technique." '822 Patent, 2:3-11.

The Johnson technique generally involves the following steps:

- collecting semen from a mammal of interest (Johnson-1992, 6:27-28);
- staining the sperm with a DNA intercalating fluorescent dye, *e.g.*, Hoechst 3342 (Johnson-1992, 6:31-33);
- determining the level of fluorescence of the stained sperm using a flow cytometer running a "viability-supporting" sheath fluid (Johnson-1992, 2:53-55, 6:38-40);
- selecting sperm with a desired amount of DNA (*e.g.*, an X- or Y-chromosome) (Johnson-1992, 4:16-18);
- collecting the selected sperm in a "viability-supporting" collector fluid (Johnson-1992, 2:56-58, 6:40-41); and
- using the collected sex-sorted sperm in artificial insemination to create embryos (Johnson-1992, Table 2, 7:6-16).

See e.g., Johnson-1992, Abstract, 6:27-7:41; Pace-Decl. ¶¶ 54-56.

The method of claim 11 closely tracks the known Johnson technique for sorting sperm. Pace-Decl. ¶ 57.

4. Pre-Sort, Sheath and Collection Fluids Used in Flow Cytometry to Sort Sperm

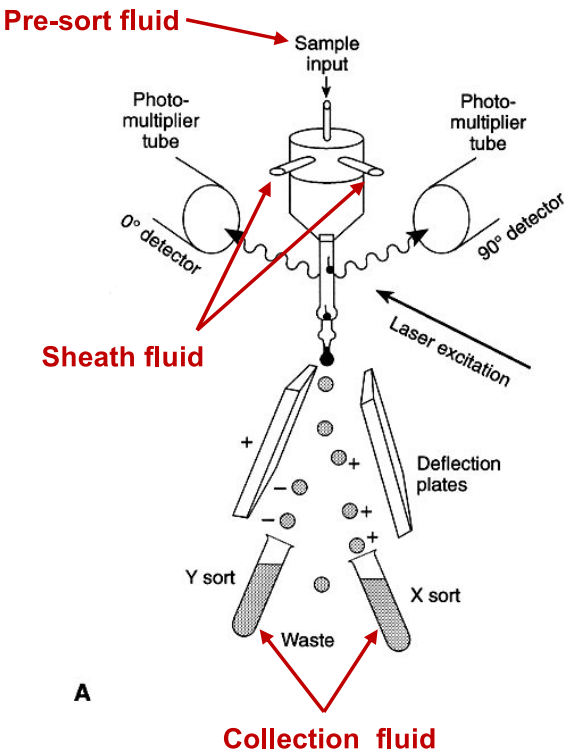
Sex-sorting by flow cytometry uses three types of fluids:

(i) a "pre-sort" or "sample" fluid containing stained sperm cells to be sorted;

(ii) a "sheath fluid" that propels the pre-sort fluid containing the cells to be evaluated through the flow cytometer, and

(iii) a "collector fluid" that is added to the collection containers that capture the sorted sperm.

Pace-Decl. ¶ 58. Fluids are also referred to as layers or streams in the literature. Pace-Decl. ¶ 59. The annotated figure below illustrates where these three fluids are used in the flow cytometer apparatus:



Cran-1996, 357, Figure 1a; Pace-Decl. ¶ 59.

Sheath fluid gets its name because it surrounds the sample layer containing the cells being evaluated but does not mix with it. Shapiro-1995, 16. As Patent Owner explained during prosecution:

> ***A basic tenet of flow cytometry provides that sheath fluid and sample do not mix in the nozzle of a flow cytometer***. Instead, ***the sheath fluid forms a coaxial outer layer which surrounds the cell containing sample***. Within the nozzle, both the core stream of sample and the outer stream of sheath fluid flow in a laminar fashion resulting in virtually no mixing (See also Appendix A - Chapter 4.6 from Practical Flow Cytometry, 4th Edition, by Howard Shapiro, particularly page 167).

'822 Patent FH, 235 (emphasis added). This phenomenon is illustrated in the textbook Patent Owner cited during examination of the '822 Patent:

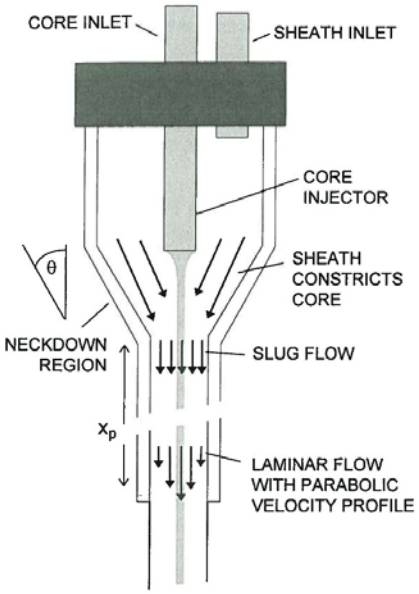


Figure 4-28. Fluid flow in a flow cytometer.

Shapiro-2003 (Ex. 1012), 167, Figure 4-37; '822 Patent FH, 235; Pace-Decl. ¶¶ 61-64.

5. Avoiding Significant Changes to an Aqueous Environment Containing Sperm Was Known to be Important

It was well understood before 1997 that a range of attributes of the aqueous environment in which collected sperm is maintained will influence their viability, including, *inter alia*, the pH, osmolality, the presence, identity and concentration of ions, the presence and identity of sugars and other compounds, and gas (e.g., CO_2 and O_2) concentration. *See generally* Salisbury-1978, 443-6. Variations in these attributes of the aqueous environment were known to cause physical changes in the

sperm cells and impair various of their metabolic functions. Thus, as Salisbury-1978 explained:

> Spermatozoa behave as osmometers and are capable of extensive changes in size depending upon the tonicity of the medium in which they exist (11, 12). Seminal plasma has an osmotic pressure of about 285 milliosmols and it is usually assumed that this represents the ideal physiological osmotic pressure. Even if this is true, however, it should not be assumed that solutions of equivalent osmotic pressure, as measured by physical tests, are isotonic with bull spermatozoa. The permeability of the cell to different substances varies. Interactions may occur between the total concentration of substances in the fluid surrounding spermatozoa, the pH of the medium, and the specific ions and nonelectrolytes present (13, 14). If the tonicity of the medium deviates considerably spermatozoa may have bent tails, swim in circles, and die. Fortunately spermatozoa are able to tolerate a moderate range of osmolarities without a reduction in fertility (15); the degree of tolerance is affected by interactions with pH and other ions present (13).

Salisbury-1978, 444.

The selection of appropriate buffer systems was also understood to be important to maintaining sperm viability. As Salisbury-1978 explains:

> **16-2.4 Buffering and pH**. Spermatozoa need protection from autotoxication due to acid products of metabolism, particularly when they are stored without refrigeration. Bull sperm motility and fertility are well preserved in egg yolk and milk extenders near neutral pH,

> although reduction of the pH to 6.5 may even be beneficial (23). The optimum pH probably varies with storage temperature and other components of the extender (13, 14). Saturation of bicarbonate media with CO_2 causes metabolic inhibition and reduces the pH to about 6.3 (24, 25, 26). This inhibition may be due in part to a reduction in pH (23).

Id., 445.

6. It Was Known that Dilution Causes Adverse Effects

It was well known before 1997 that dilution of the environment in which sperm cells are maintained can cause stress on the cells, and therefore agents were routinely added to counter those deleterious effects. *See, e.g.*, Salisbury-1978, 446-7 ("Studies have shown that extensive "dilution" of the spermatozoa with simple solutions depresses motility (41) but that the addition of amino acids and macromolecules such as egg albumin or casein minimizes this effect."); Johnson-1992, 2:56-61; 12:38-41; 13:56-60.

Dilution occurs during flow cytometry when the pre-sort (sample) and sheath fluids mix in the collection container. As explained in § IV.A.4, the sheath and sample fluids will remain separate until they combine in the collection unit. Shapiro-1995, 16; Johnson-1992, 4:51-5. When that occurs, the sheath fluid will dilute the collection environment containing the sperm cells and expose them to the agents in the sheath fluid. Johnson-1992; 10:43-48; '822 Patent FH, 235; Pace-

Decl. ¶¶ 78-79. The sheath fluid can dilute the sample fluid in the collection unit by 70-100 fold. '822 Patent FH, 236; Pace-Decl. ¶ 80.

Johnson-1992 not only identified the problem of dilution of the sorted sperm in the collection unit, but taught a solution for it – add an extender solution to the collection unit that will maintain the viability of the sorted sperm:

> Dilution of sperm as occurs in sorting tends to reduce viability of the cells. To overcome this problem, sperm were collected in test egg yolk extender [Graham et al., J. Dairy Sci. 55: 372 (1972)] modified by adjusting the pH and adding a surfactant.

Johnson-1992, 4:51-8; *see also id*., 10:38-48. The "test egg yolk extender" was the extender solution used by Johnson-1992 in its examples.

Other publications by Johnson before 1997 similarly explain this problem and its solution:

> After staining, the sperm are sorted into tubes containing 50 μL TEST-yolk medium (Johnson et al. 1989; Johnson 1991). The TEST-egg yolk extender into which the sperm are received is critical to their survival after sorting because of the excessive dilution caused by the presence of the sheath fluid in the flow sorting process.

Johnson-1995 (Ex. 1013), 900.

Thus, before 1997, it was well known that the sheath fluid will dilute the agents and sperm in the collection container of a flow cytometer, and that steps

need to be taken to mitigate the impact of that dilution after the sperm have been sorted. Pace-Decl. ¶ 82.

7. Advances in High Speed Flow Cytometry Made Commercial Scale Sorting Feasible

While Johnson demonstrated that one could sex-sort sperm and achieve >90% purity of single sex sperm samples that would successfully impregnate females, he acknowledged the systems being used at that time were too slow for commercial applications. Johnson-1989, 203 ("…several factors mitigate against widespread application of this methodology at the present time: (1) The limitation on number of sperm that can be sorted in a reasonable period of time (about 3.5 x 10^5 per hour) eliminates use of this procedure for producing sexed semen for standard cervical artificial insemination in most mammals…."). Until the early 1990's, flow cytometers continued to have relatively slow sort rates (~3.5 x 10^5 cells per hour or <1000 cells per second). Johnson-1989, 203; Pace-Decl. ¶¶ 83-84. Because the viability of sperm cells diminishes over time once the fresh ejaculate is produced, even with extenders, these slow sort rates decreased the yield of viable sperm, and made sex-sorting of sperm not commercially viable. Pace-Decl. ¶ 84.

By the mid-1990's, advances in high-speed flow cytometers "enabled logistically simpler experiments and much faster sorting rates" that made sex sorting of sperm commercially viable. Seidel-1998 (Ex. 1014), 22; *see also* Kling-

1997 (Ex. 1015), 15 ("HIGH-SPEED SORTER: Cytomation's MoFlo (modular flow) cytometer reportedly can perform multiparameter cell sorting at rates exceeding 25,000 cells per second."); Pace-Decl. ¶ 85. As the '822 Patent thus acknowledges:

> One of the aspects of flow cytometry which is particularly important to its application for sperm sorting is the high speed operation of a flow cytometer. Advances have been particularly made by the flow cytometers available through Cytomation, Inc. under the MoFlo® trademark. These flow cytometers have increased sorting speeds extraordinarily and have thus made flow cytometry a technique which is likely to make feasible the commercial application of sperm sorting (among other commercial applications).

'822 Patent, 6:59-67. Thus, before 1997, flow cytometry-based sex sorting of sperm had become commercially viable. Pace-Decl. ¶ 87.

8. Ordinary Artisans Understood the Need to Optimize Conditions When Sorting Sperm to Improve Yield

Ordinary artisans recognized that optimizations beyond sorting speed were desirable and could be realized by adjusting the conditions used during the sorting, handling and use of the sperm. Pace-Decl. ¶¶ 90-92. The '822 Patent reflects this well understood fact, observing that "…it has always been known that the sperm themselves are extremely delicate cells." '822 Patent, 2:45-7. The '822 Patent also lists factors that an ordinary artisan knew before 1997 would decrease yields of viable sperm:

> The sensitivities range from ***dilution problems*** and the flow cytometer's inherent need to isolate and distinguish each cell individually as well as the ***pressure and other stresses which typical flow cytometry has***, prior to the present invention, ***imposed upon the cells or other substances that it was sorting***.

Id., 2:56-66 (emphasis added). Pace-Decl. ¶ 93.

Before 1997, an ordinary artisan thus would have understood the importance of selecting the conditions and makeup of the solutions used in the handling of sperm from the point of collection, during sorting and continuing up to when the sperm is used to inseminate an animal, in order to maximize yield of viable sperm. Pace-Decl. ¶ 94. Indeed, it was well established that doing so was critical to achieving a high fertility rate, which, in turn, enabled pregnancies and the ongoing production of milk for the dairy farmer customer. Pace-Decl. ¶ 94; Johnson-1992, 4:19-22.

B. Conditions for Maximizing Sperm Health Were Well Known Before 1997

Well before 1997, extensive experience had been gained in identifying buffers and other reagents that maximize sperm health (*e.g.*, viability, motility, and fertility) during the collection and processing of sperm for use in artificial insemination. Pace-Decl. ¶¶ 95-96. Like conventionally prepared sperm samples, sex sorted sperm are relatively useless even for research purposes if the produced sperm are incapable of fertilization. Johnson-1992, 4:19-22 ("It is, of course, of

critical importance to maintain high viability of the intact sperm during the sorting process and during storage after sorting but prior to insemination."); Pace-Decl. ¶ 95.

The incentive to maximize yields of viable sperm was particularly well-understood in the commercial setting – if the quantity of viable sperm in a sample being used to inseminate an animal are too low, use of those samples becomes commercially impractical (e.g., they will produce low rates of fertilization, waste resources and time, and cause an interruption to the on-going milk supply process from the dairy farmer). Pace-Decl. ¶ 94. Consequently, it was well understood before 1997 that achieving even small (1 to 3%) increases in the quantity of viable sperm in a sperm sample was important. Pace-Decl. ¶ 97. The '822 Patent acknowledges this, observing that the problem of achieving "artificial insemination with a high success rate is one of a statistical nature in which a multitude of factors seem to interplay." '822 Patent, 3:23-35.

The need to achieve even incremental increases in yield of viable bovine sperm spawned decades of research before 1997. Pace-Decl. ¶ 98. That research showed that optimal choices for sperm from one species would not necessarily be the optimal choices for sperm from a different species. Pace-Decl. ¶¶ 98-99. Consequently, an extensive amount of guidance on the optimal buffers and agents

to use in the handling of different types of sperm cells is found in the public literature before 1997.

1. Extender Solutions Were Used to Maintain Viability of Collected and Processed Sperm

Producing a sample of sperm for use in insemination – whether conventional unsexed sperm or sexed sperm using flow cytometry – starts with dilution of the raw semen ejaculate. This is necessary because of the high sperm concentration in the ejaculate and the presence of semen. Salisbury-1978, 442. Raw ejaculate is conventionally diluted in a medium called an "extender," which extends the sperm's viability outside of the body as it is diluted. Salisbury-1978, 442. Extension is necessary to preserve the fertility of the sperm and to increase the total volume of fluid containing the sperm so that a precise dose of sperm can be packaged and used for insemination. Salisbury-1978, 442. In this way, the raw ejaculate can be "extended," so as to maximize the use of the ejaculate sperm cells and minimize waste. Pace-Decl. ¶¶ 100-102.

Extender solutions were also routinely used throughout the processing of collected sperm. For example, if samples are to be frozen or thawed, extender solutions must be employed to lessen the impact on the cells. Salisbury-1978, 442, 446, 498-499. In the context of flow cytometry-based sexing of sperm, extenders were added to the collection containers to lessen the impact of dilution of the

sample by the sheath fluid. Johnson-1992, 4:51-8, 6:40-7; Johnson-1989, 200; Johnson-1991, 310.

Typical extenders known before 1997 included a buffer, an acid to adjust pH, a sugar and egg yolk. Pace-Decl. ¶ 105; Salisbury-1978, 444-6. Although the choice of buffer, pH modulator, sugar and other components varied, egg yolk in particular was recognized as being key to protecting sperm cells. Pace-Decl. ¶ 105. Before 1997, many different extender formulations had been evaluated to determine their relative effectiveness in maintaining the viability of different species of sperm, and a small number that provided optimal results for particular types of sperm (e.g., bovine) had become well known and widely used before that date. Pace-Decl. ¶ 106; Salibury-1978, 444-446.

2. The Benefits of Citric Acid in Solutions Used in Processing Bovine Sperm Samples Were Well Known

A handful of extender formulations containing citric acid (e.g., Tris, CUE, and citrate-egg yolk) were commonly used during the collection, processing and use of bovine sperm in artificial insemination. Citric acid also was commonly used to modulate or alter pH in bovine semen extenders prior to December 1997. Pace-Decl. ¶ 108. For example, Tris based buffers were commonly titrated with citric acid. Salisbury-1978, 455 ("Optimum sperm survival was obtained in 0.2 molar Tris-yolk extender, adjusted with citric acid to pH 6.75.").

Conventional bovine extenders containing citric acid remained the industry standard in December 1997 and thereafter. Pace-Decl. ¶ 109. Certified Semen Services (CSS), Inc. is a wholly owned subsidiary of the National Association of Animal Breeders (NAAB), sets standards of performance and conduct in the livestock artificial insemination. Pace-Decl. ¶ 109. In January 1997, CSS published the "Minimum Requirements for Disease Control of Semen Produced for AI." ("CSS-1997") (Ex. 1016). That publication, *inter alia*, recommended five extenders for bull semen, three of which are prepared using citric acid, sodium citrate, or both. CSS-1997, 6-7. Pace-Decl. ¶ 109.

In addition, research done before 1997 compared various types of buffer systems and found that those containing citric acid performed as well or better in preserving viability of bovine sperm than many other buffer systems. That research compared the effects of different organic and inorganic acids (including different counter ions of the acids) on the viability of fresh and frozen/thawed samples of bovine sperm, and placed buffer systems including a citric acid at or near the top of the list for maximizing bovine sperm viability. Garcia-1989-III, 1040-3; *Id.*, 1046 ("Sperm motility of fresh and frozen thawed samples was high in potassium phosphate buffers and was not different than motility in samples containing Na citrate."). For sperm that had been frozen and thawed, Garcia-1989-III reported that "[i]norganic salts of phosphates, carbonates or chloride provided

significantly less protection to the cells than the control extenders with Na citrate ($p<0.05$)" and that "citrate, tartrate and oxalate salts provided superior ($p<0.05$) protection to the cells than salts of succinate, acetate or formate." Garcia-1989-III, Abstract; 1046.

An ordinary artisan before 1997 thus would have viewed use of buffer systems including a citric acid in the handling of bovine sperm to be one of a handful of preferred options and to be superior to other buffer systems for that type of sperm, including those based on phosphates. Pace-Decl. ¶¶ 112-115.

V. THE CONTESTED PATENT

A. Effective Filing Date

The '822 Patent claims priority to a series of applications, the earliest of which is dated December 31, 1997 (i.e., U.S. Application No. 09/001,394). Each of the prior art references used in this proceeding was published more than one year prior to that date and is prior art under 35 U.S.C. § 102(b).

B. Prosecution History of the '822 Patent

The '822 Patent issued from U.S. Application 13/764,408. During examination, the Patent Owner contended the prior art did not teach use of a citric

acid in the sheath fluid.[1] *Id*., 235. Patent Owner did not dispute that the prior (e.g., Johnson-1992) taught use of a pre-sort media including a citric acid ("Johnson may be characterized as describing a pre-sort staining step with includes citric acid…"). *Id*. Patent Owner likewise acknowledged the known value using citric acid in sperm cell extenders, thereby recognizing the ordinary artisan knew of the value of using pre-sort and collection fluids that include a citric acid. *Id*., 237. But Patent Owner contended it was the first to appreciate that dilution of citrate in the collection fluid by sheath fluid was a "problem," asserting:

> Applicants for the first time appreciated a new problem with respect to sperm sorting. In particular, droplets formed from the coaxial stream described above may contain between 70 and 100 times more sheath fluid than [the] sample. Even in the event droplets are sorted into a container containing a volume extender with citrate, the sperm cells remain largely diluted in sheath fluid. This problem is alluded to as the "dilution problem" referenced in Applicant's Original Specification at page 3, line 27, and expanded upon at page 11, line 20 carrying on to page 12, line 29.

Id., 236-7. After an interview, Patent Owner's amended claim 77 was allowed and issued as claim 11 of the '822 Patent. '822 Patent FH, 139-40.

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[1] The applicants made this argument for claims requiring "citrate" in the sheath and for claims requiring "citric acid" in the sheath.

As explained elsewhere (*see* § IV.A.6), Patent Owner plainly was not the first to recognize that the sheath fluid dilutes the collection fluid and thereby can cause stress on sperm in the collection unit. Likewise, it was not the first to solve this problem – Johnson-1992 solved it by teaching inclusion of an extender in the collection container and use of a sheath fluid that maintains the viability of the sperm. Selecting a buffered solution including citric act to use as the sheath fluid also was an obvious choice for bovine sperm – those buffer systems had been shown to be effective in protecting bovine sperm from the stress of dilution and handling incidental to its use in artificial insemination. Pace-Decl. ¶ 110.

C. The Contested Claim – Claim 11

The ’822 Patent concerns flow cytometry systems and methods used to sort sperm. The sole contested claim is claim 11, which reads:

> A method of producing at least one sexed embryo comprising:
>
> producing a stream containing sperm cells, wherein the stream comprises sperm cells from a cell source surrounded by sheath fluid, wherein the sheath fluid surrounding the sperm cells includes a citric acid;
>
> identifying X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in the stream;
>
> collecting X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in at least one collector having a collector fluid which includes a citric acid; and

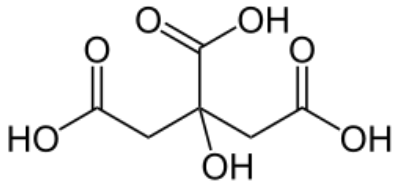
fertilizing at least one egg with the collected sperm cells to form at least one sexed embryo.

D. Proposed Constructions

The language used in claim 11 employs terms that are not expressly defined in the patent, much less in a manner that would cause them to be read to have a meaning at odds with the ordinary meaning of those terms. Those terms instead are used with their ordinary meaning.[2] To avoid doubt, proposed constructions for certain of these terms are proposed by Petitioner.

1. "includes a citric acid"

The term "citric acid" has a well-defined meaning – it is a compound having the formula $C_6H_8O_7$ and the following chemical structure:



Citric acid is a tricarboxylic acid. *See* Masterton-1977 (Ex. 1017), 392. Citric acid forms salts with many different counter ions (e.g., Na^+, K^+). Salts and esters of citric acid are generally referred to as "citrates." Pace-Decl. ¶ 168. Citric acid behaves as an acid in an aqueous environment, forming a dynamic equilibrium of molecules in a protonated (i.e., AH) and unprotonated (i.e., A-) state.

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[2] The '822 Patent expired in December of 2017.

Masterton-1977, 453; Pace-Decl. ¶ 167. The proportion of protonated and unprotonated forms of citric acid in an aqueous solution is influenced by many factors, including the concentration of citric acid in solution, overall pH and the presence of cations. Masterton-1977, 456-7; Pace-Decl. ¶ 167.

The specification describes production of a sheath fluid by adding a citrate (i.e., sodium citrate dihydrate) to water, but not by adding citric acid to water.[3] '822 Patent, 8:50-9:5 (sheath); *id.*, 11:14-12:8 (collection). It also describes production of a collection fluid by adding both citric acid and a citrate to water. *Id.* at 13:38-50. The claims encompass both of these solutions because each "includes a citric acid" – each will contain some number of citric acid molecules when sodium citrate or citric acid is added to water. Pace-Decl. ¶ 171.

Importantly, nothing in the claim language or patent disclosure requires use of a particular concentration of citric acid. Likewise, nothing restricts how a solution "including a citric acid" must be prepared. And nothing in the claim language excludes a solution containing counter ions (e.g., Na^+) or other molecules in addition to citric acid molecules. Thus, the claims would by their plain language encompass, *inter alia*, a sheath or collection fluid produced by adding sodium

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[3] The named inventor testified there is no functional consequence of using a citrate rather than citric acid. Seidel-Tr., 81:12-83:15; 84:1-7.

citrate to water as shown in the patent disclosure. *See, e.g.*, '822 Patent, 8:50-9:5 (sheath); *id.*, 11:14-12:8 (collection).

Nothing in the prosecution history of the '822 Patent raised an issue affecting patentability based on the presence or absence of agents besides citric acid in the sheath or collection fluids or how the solution is produced. There is thus no basis for narrowing the meaning of "includes a citric acid" based on the prosecution history.

Consequently, as used in the patent claims, the phrase "*includes a citric acid*" should be read as meaning that the solution comprises citric acid molecules and may contain other agents or counterions.

2. "the sheath fluid surrounding the sperm cells"

Claim 11 specifies "wherein *the sheath fluid surrounding the sperm cells* includes a citric acid."

As used in the specification, "sheath fluid" has its conventional meaning – it is a fluid introduced into a flow cytometer that surrounds the sample layer and propels it through the flow cytometer. *See, e.g.*, '822 Patent, 6:55-62. The specification does not provide an alternative definition of "sheath fluid" either implicitly or explicitly.

Nothing in the specification requires use of a particular makeup of sheath fluid. Instead it explains that a skilled person will select the makeup of the sheath

fluid based on the type of sperm cell and the particular cell sorting situation at issue. '822 Patent, 7:65-8:25, 8:41-56; Pace-Decl. ¶ 172. For example, the specification describes preparation of two different sheath fluids using different sets of agents, one for use in bovine sperm and one for equine. For the first, disodium citrate dihydrate was added to a quantity of water. '822 Patent, 8:50-9:5 (sheath). For the second, a number of agents including HEPES were added to water. *Id*., 9:6-33. Notably, claim 11 is not restricted to any particular type of sperm cell, and does not recite any other factors that would indirectly limit the makeup of the sheath fluid, other than it include at least a citric acid. Finally, nothing in the prosecution history of the '822 Patent suggests that an issue of patentability turned on the makeup or characteristics of the "sheath fluid" other than it include a citric acid.

There is thus no basis for restricting meaning of "sheath fluid" to require or exclude the presence of other agents beyond citric acid, or to require a functional property for the sheath fluid. Consequently, the phrase "*sheath fluid*" means "a solution introduced into a flow cytometer that surrounds and propels a sample through the flow cytometer."

3. "collection fluid"

The '822 Patent does not explicitly or implicitly define "collection fluid." As used in the disclosure, it has its conventional meaning of being a fluid present

in a collection container that receives sperm cells after they are sorted by the flow cytometer. *See, e.g.*, '822 Patent, 6:32-3; 10:38-41.

For example, the '822 Patent explains that collection fluids are added to collection containers before sperm are sorted into them. The '822 patent also generally indicates that the purpose of using a collection fluid is to minimize stress on the cells. *Id.*; *see also id.*, 10:42-11:3. One such stress is the known adverse effect of dilution of the sperm cells in the collection container that occurs during the normal operation of the flow cytometer. *Supra* § IV.A.6; I.C.6.

The '822 Patent acknowledges the known dilution phenomenon and the typical way ordinary artisans mitigate its impact – by adding agents to yield a desired aqueous environment in the collection container during sorting:

> Furthermore, since the initial chemical substance content can be varied (for instance the percent egg yolk content in the citrate may be varied up or down), likewise the starting collection fluid environment or various volumes may also be varied so that the ending result is the same. Thus, prior to commencing the sorting process, the collector fluid exists with a six percent egg yolk content in the citrate solution and after completion of the sorting event the collector fluid with the sex-specific sperm may result in a two percent egg yolk content in the citrate solution similar to the initial nutrient content.

'822 Patent, 11:3-14. In other words, the '822 patent recognizes that the makeup and identity of the collection fluid will vary, and will not be fixed, during the process of sorting sperm.

Nothing in the prosecution history addressed the identity of the collection fluid or suggested an issue of patentability turned on its makeup.

Consequently, "collection fluid" means simply "a fluid added to a collection container that receives sorted sperm cells from a flow cytometer."

4. No Other Limitations Imposed by Claim Language

Claim 11 is not limited with respect to the species of cells being sorted. Instead, it refers simply to "sperm cells." The specification provides examples of sorting two different types of sperm – bovine and equine – but does not describe its techniques in a way that would limit them to sorting one species of sperm cell.

Claim 11 also does not impose quantitative thresholds for the efficiency of sorting, on the percentage of sperm cells remaining viable at the different stages of the sorting process, or the effectiveness of successful insemination. Instead, all it specifies is that the claimed method result in fertilization of "at least one egg with the collected sperm cells" that have been sexed.

VI. DETAILED REASONS WHY CLAIM 11 IS UNPATENTABLE

As the Board has instructed, an obviousness ground must set forth reasoning and evidence according to criteria articulated in *Graham v. John Deere* and

subsequent cases. First, it must describe and explain what the teachings of the prior art at issue would have conveyed to the person of ordinary skill. Second, it must identify the differences between the primary reference and the disputed claim. Third, it must explain why the claim in view of those differences would have been obvious to an ordinary artisan, explaining, *inter alia*, why the ordinary artisan would have combined the references and how they together would have made the claimed subject matter obvious. Finally, the petition should address any relevant and known secondary considerations. *See Praxair Distrib., Inc. v. INO Therapeutics, Inc.*, Case Nos. IPR2015-00522, -00524, -00525, -00526, slip op. at 16–17 (PTAB July 29, 2015); Coalition for affordable Drugs V LLC v. Hoffman-LaRoche Inc., Case No. IPR2015-01792 (PTAB March 11, 2016), Paper 14 at 35.

Each of these required elements is presented below, which demonstrates that claim 11 would have been obvious based on Johnson-1992 in view of Salisbury-1978 and further in view of Garcia-1989-III.

A. Person of Ordinary Skill in the Art

A 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 of experience in a field relating to sperm cell physiology. Pace-Decl. ¶ 41.

The Patent Trial and Appeal Board has previously found Dr. Marvin M. Pace, who has provided an expert declaration with this petition, a person of

ordinary skill in the art in an *Inter Partes* Review proceeding involving a patent directed to methods of freezing sex sorted sperm. *ABS Global, Inc. and GENUS PLC v. XY, LLC.*, No. IPR2014-01550, Paper 25 at 6-7.

B. Relevant Teachings of the Primary Reference – Johnson-1992

Johnson-1992 issued on August 4, 1992, more than one year prior to the earliest effective filing date of the ’822 Patent. Johnson-1992 is prior art to at least claim 11 of the ’822 Patent under 35 U.S.C. § 102(b).

The ’822 Patent disclosure not only admits Johnson-1992 is prior art, but credits it as pioneering the “Johnson technique” which the claimed methods of the ‘822 patent use. As ’822 patent explains:

> At present, 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. This technique appeared possible as a result of advances and discoveries involving the differential dye absorption of X- and Y-chromosome bearing sperm. This was discussed early in U.S. Pat. No. 4,362,246 and significantly expanded upon through the techniques disclosed by Lawrence Johnson in ***U.S. Pat. No. 5,135,759***. The Johnson technique of utilizing flow cytometry to separate X- and Y-chromosome bearing sperm has been so significant an advancement that it has for the first time made the commercial separation of such sperm feasible.

’822 Patent, 1:64-2:10 (emphasis added).

Johnson-1992 discloses the widely cited work of Lawrence Johnson describing the initial reports of use of flow cytometric sex sorting of sperm based on differences in DNA content and use of sorted sperm to impregnate mammals (i.e., rabbits and swine). Johnson-1992, 1:10-13; Pace-Decl. ¶¶ 117-118. Johnson-1992 demonstrated, *inter alia*, "the separation, by flow sorting, of intact, viable X and Y chromosome-bearing rabbit and swine sperm populations based on relative DNA content; surgical insemination of the sorted sperm into does; and the subsequent birth of sexed offspring with a phenotypic sex ratio consistent with predictions based on the relative DNA content of the sorted sperm populations." *Id*., 2:64-3:3.

Johnson-1992 describes use of the three fluid environments in its flow cytometry environment: (1) a pre-sort (or sample) fluid environment, (2) a sheath fluid environment, and (3) and a collector fluid environment. Pace-Decl. ¶ 119. Johnson-1992 teaches that each of these fluids is to be selected so as to achieve the common object of the invention of "maintaining the viability of" sperm cells. As it states in the Summary of the Invention section:

> It is a further object of this invention to teach a method of staining the DNA of mammalian sperm ***while maintaining viability of the sperm***.

> It is a further object of this invention to provide a sheath fluid adapted to be used in a cell-sorting apparatus ***while maintaining viability of sperm cells***.
>
> It is a further object of this invention to provide a collecting fluid capable of ***maintaining the viability of sorted sperm cells***.

Johnson-1992, 2:50-8 (emphasis added).

Johnson also notes the importance of using fluids that will maintain the viability of the intact sperm during and after the flow cytometry-based sorting of the sperm cells, stating:

> It is, of course, of critical importance to ***maintain high viability of the intact sperm*** during the sorting process and during storage after sorting but prior to insemination.
>
> Of the factors involved in ***maintaining sperm viability***, the method of staining, the sheath fluid, and the collecting fluid have been found to be especially important.

Id., 4:19-26 (emphasis added).

An ordinary artisan would have understood these observations to be indicating that the chemical makeup of the three fluids should be selected to not only be suitable for the particular type of sperm being sorted, but to minimize stresses on the sperm cells caused by changes to the nature of the aqueous environment the sperm are exposed to before, during and after sorting. Pace-Decl. ¶ 122. In particular, from these observations in Johnson-1992 and his or her

personal experiences, the ordinary artisan would have sought to minimize changes in the makeup of the fluid environment used before, during and after sorting of sperm that would affect the isotonicity, osmolality, pH, salt concentration and other aspects of that fluid environment. Pace-Decl. ¶ 123. The ordinary artisan also would have recognized the simplest way to maintain consistency of these fluid environment would be to use the same buffering systems in the pre-sort, sheath and collection fluids. Pace-Decl. ¶ 124.

Johnson-1992 also describes the general requirements of sheath fluids, explaining:

> Sheath fluid used in sorting cells must be electrically conductive and isotonic. A concentration of 10 mM phosphate buffered saline provided the necessary electrical properties, and 0.1% bovine serum albumin was added to enhance sperm viability by providing protein support for metabolism and viscosity for the sperm. The sheath fluid must be free of sugars and excess salts.

Johnson-1992, 4:44-50. These observations are consistent with generally held understandings before 1997 that the isotonicity, osmolality, salt concentration and pH of the fluids (among other factors) in which sperm are kept should be selected to maintain the viability of those cells, and that those factors will be tailored to the type of sperm being sorted. Pace-Decl. ¶ 132. *See also* § IV.A.5.

Johnson-1992 also explains the sheath fluid will dilute the environment of the sperm cells in the collection unit, that this dilution can cause stress on the sperm cells, and to mitigate that stress, an extender should be added to the collection unit:

> Dilution of sperm as occurs in sorting tends to reduce viability of the cells. To overcome this problem, sperm were collected in test egg yolk extender [Graham et al., J. Dairy Sci. 55: 372 (1972)] modified by adjusting the pH and adding a surfactant. Details of the composition of the extender are shown in Example 1. The surfactant is believed to enhance capacitation of the sperm prior to fertilization.

Johnson-1992, 4:51-8.

The Graham et al. paper referenced by Johnson-1992 ("Graham-1972") (Ex. 1018) describes a variety of buffered extender solutions suitable for use in dilution, extension and handling of sperm for use in artificial insemination. Table 4 of Graham summarizes their findings, reporting that a variety of zwitterionic buffers performed well in maintaining motility of the sperm, and that sodium citrate provided better protection relative to phosphate in their testing on effects on motility of sperm:

TABLE 4. Differences in percentage motile spermatozoa and in glutamic oxalacetic transaminase (IU/10^9 spermatozoa) release by buffer including all titration bases and treatments.

	Principal buffers							Other buffers		
	TES	BES	MOPS	TRICINE	HEPES	PIPES	MES	NaCitrate	TrisHCl	Phosphate
Motility[1] (%)	57.8a	55.4ab	55.3b	54.7b	54.0b	53.6bc	51.3c	51.0c	45.2d	29.1e
GOT[2]	263.6cde	255.3e	267.9cd	306.2a	284.1c	269.4c	269.8c	257.7de	286.0b	248.7f

[1] n = 80 motilities per mean for the principal buffers and 20 per mean for the other buffers.
[2] n = 100 GOT values per mean for the principal buffers and 25 per mean for the other buffers.
a,b,c,d,e,f—Means not followed by the same letter in the same horizontal line are significantly different at $P<.05$.

Graham-1972, 377. An ordinary artisan would have consulted Graham-1972 for further guidance on collection fluids to use given the manner with Johnson-1992 cites to it. Pace-Decl. ¶¶ 127-130.

Johnson-1992 also explains that the sheath fluid should not contain other agents, such as sugars or excess salts. This would have been made sense to the ordinary artisan, given that it was well known that the sheath fluid does not interact with the sperm cells until it combines with the sample fluid in the collection unit. '822 Patent FH, 235. The ordinary artisan thus would have recognized there was no need to include additional agents in the sheath fluid to stabilize the sperm cells, such as agents found in extenders like sugars or egg yolk. Pace-Decl. ¶ 131.

The ordinary artisan also would have recognized the connection between the observations in Johnson-1992 that (i) the sheath fluid will dilute the collection fluid and (ii) the makeup of the sheath fluid and the collection fluid should be selected to maximize the viability of the sperm cells. That connection would have led the ordinary artisan to select a sheath fluid that will not impair the ability of the collection fluid to minimize stress on the sorted sperm, such as by changing the

osmolality or isotonic nature of that environment (e.g., by introducing different acids or counter ions, or by materially altering concentrations of the buffer agents). Pace-Decl. ¶¶ 132-133.

Johnson-1992 also describes examples of buffers to be used in each of the recited fluid environments. For the pre-sort fluid, Johnson describes use of a TRIS-citric acid-based buffer solution. Johnson-1992, 6:29-31 ("[t]he semen was diluted with Tris buffer, pH 6.9…."). [4] For the sheath fluid, Johnson-1992 indicates that a 10 mM phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (BSA) was used. Johnson-1992, 6:38-40. And for the collection fluid, Johnson-1992 describes use of the "TEST-egg yolk" extender having the following composition:

> The composition of the extender was N-tris(hydroxymethyl)-methyl-2-amino ethane sulfonic acid, 2.16 g; tris hydroxymethyl aminomethane, 0.51 g; dextrose, 0.1 g; streptomycin sulfate, 0.13 g; penicillin G, 0.08 g; egg yolk, 12.5 ml; Equex STM (Nova Chemical Sales, Scituato, Mass.), 0.5%; and distilled water, 50 ml.

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[4] Johnson-1992 does not explicitly identify all of the components of the "Tris buffer" that was used. In an earlier publication discussing the same work, Johnson explained that the "Tris buffer" contains "tris(hydroxymethyl)aminomethane, 2.52 g; D glucose, 1.04 g; **citric acid, 1.28 g**, pH 6.9…." Johnson 1989, 200.

Johnson-1992, 6:42-7. The "TEST" buffer is the Tris-ethane sulfonic acid buffer that was described by Graham et al. *See* Graham-1972, 373.

Johnson-1992 also reports obtaining rabbit and pig offspring from sorted sperm obtained from his process, demonstrating proof of concept. *See* Johnson-1992, 7:1-18 ("EXAMPLE 2"); *id.*, 35-41 ("EXAMPLE 4"); *see also* Tables I and II.

Johnson acknowledged in contemporaneous publications that the slow sorting speeds of his experimental systems limited their commercial application, but also anticipated improvements to solve that problem, and that this system would be useful in cattle breeding. As he stated in a 1989 paper:

> The results described here represent a significant advance toward the goal of sex preselection for mammals. However, several factors mitigate against widespread application of this methodology at the present time: (1) The limitation on number of sperm that can be sorted in a reasonable period of time (about 3.5×10^5 per hour) eliminates use of this procedure for producing sexed semen for standard cervical artificial insemination in most mammals. (2) The increased embryo mortality presumed to be related to the presence of the fluorochrome on the DNA. (3) The cost of the modified flow cytometer/sperm-sorting instrumentation (approximately $250,000). However, none of these factors appear to represent insurmountable difficulties. In fact, the current procedure might be effectively used in conjunction with in vitro fertilization, especially with respect to cattle.

Johnson-1989, 203; *see also, e.g*., Johnson-1991, 314 ("Therefore, our current efforts center around increasing the speed of sor[ting] by 2 to 5 times so as to use sorted sperm more efficiently in conjunction with bovine and porcine in vitro fertilization and embryo transfer techniques (unpublished).").

C. Comparison of Claim 11 to Johnson-1992

Claim 11 is indistinguishable from the process for sorting sperm described in Johnson-1992 with one exception: the examples in Johnson-1992 did not use sheath and collection fluids that "include a citric acid." Pace-Decl. ¶ 195. A comparison of each element of claim 11 to Johnson-1992 is provided below.

1. "[a] method of producing at least one sexed embryo"

Johnson-1992 discloses the insemination of mature New Zealand White does (female rabbits) with sex sorted sperm and the successful production of offspring rabbits. Johnson-1992, 7:21-33, Table II. Johnson also describes obtaining "[t]wo litters (18 pigs) from surgically inseminated boar semen" using the techniques employed for sorting rabbit semen in Examples 1 to 3, and that the sexed semen "produced 88% females from X-sorted sperm and 67% males from Y-sorted sperm." *Id*., 7:38-41.

An embryo is an early stage of development of a multicellular diploid eukaryotic organism that develops from a zygote (the single cell resulting from the fertilization of the female egg cell by the male sperm cell). Salisbury-1978, 136-

138; Pace-Decl. ¶ 200. Claim 11 also is not restricted to any species of sperm or embryo. By describing the successful breeding of offspring of rabbits and pigs, Johnson-1992 necessarily describes a method of producing at least one sexed embryo, which must exist before mature forms of rabbits and pigs exist.

2. "producing a stream containing sperm cells, wherein the stream comprises sperm cells from a cell source surrounded by sheath fluid, wherein the sheath fluid surrounding the sperm cells includes a citric acid"

Johnson-1992 discloses flow cytometric sorting of sperm derived from various mammals. *See, e.g.*, Johnson-1992, 5:4-6 ("Rabbit semen was collected, diluted, and stained with a fluorochrome dye. The unsorted sperm sample from each animal would be understood to be "*a cell source*" as it is a source of sperm cells to be sorted. Pace-Decl. ¶ 202.

In Example 1, Johnson-1992 illustrates sorting a sample of rabbit sperm in which the sperm were mixed with a buffer (TRIS), stained, and then sorted using "a modified EPICS V flow cytometer/cell sorter." Johnson-1992, 6:27-68; see also *id*., 7:36-8 (swine sperm sorted using same method). An earlier Johnson publication explained this was an EPICS V flow cytometer modified in three ways: "1) beveling the sample injection tube tip; 2) replacing the forward angle light scatter detector with a fluorescence detector; and 3) transferring the collected fluorescence from the added fluorescence detector to an existing photomultiplier with an optical fiber bundle." Johnson-1986 (Ex. 1019), 268.

Standard flow cytometers, such as the EPICS V flow cytometer, work according to the known principles of fluid dynamics. Pace-Decl. ¶ 205. Generally, a sample containing cells is injected into the flow cytometer. "Sheath fluid" is then introduced which propels that sample of cells through the instrument. The sheath fluid forms a "central core" around the sperm sample but does not significantly mix with the sperm sample. '822 Patent, 5:53-6:4; Shapiro-1995, 16. This phenomenon is termed laminar flow, and was well understood by ordinary artisans before 1997. Shapiro-1995, 16; *supra* § IV.A.4; § V.B. Using conventional techniques, the sperm cells are oriented to flow in a "single file" within the central core. Johnson-1992, 3:14-4:18; Johnson-1986. Johnson-1992 refers to use of a sheath fluid in the sperm sorting process. Johnson-1992, 4:23-6, 4:44-50, 6:38-41.

An ordinary artisan would have understood this description of the examples in Johnson-1992 as describing the production of a stream containing sperm in which sheath fluid surrounds the sperm. Pace-Decl., ¶¶ 58, 119.

Johnson-1992 indicates that the sheath fluid should be "adapted to be used in a cell-sorting apparatus while maintaining viability of sperm cells." Johnson-1992 2:53-55. In The sheath fluid used in Johnson-1992 was a phosphate buffered saline (PBS) solution containing 0.1% bovine serum albumin (BSA). Johnson-1992, 4:45-50, 6:38-41. BSA was a commonly used additive in solutions used to handle sperm cells. Pace-Decl. ¶ 208.

Johnson-1992 thus describes flow cytometry methods that produce a stream comprising sperm cells surrounded by a sheath fluid that is selected to maintain the viability of the sperm. Pace-Decl. ¶ 209. Johnson-1992, however, does not expressly describe use of a sheath fluid that includes a citric acid in its flow cytometry-based sperm sorting examples.

3. "identifying X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in the stream"

Johnson-1992 describes processes that discriminate between sperm cells containing an X-chromosome and a Y-chromosome based on the difference in fluorescent intensity. *See, e.g.*, Johnson-1992, Abstract, 2:47-9, 2:64-3:3, 6:27-68, claim 1; Pace-Decl. ¶ 211-212. For example, claim 1 of Johnson-1992 is directed to "[a] method for sorting intact, viable, mammalian sperm into X- and Y-chromosome-bearing populations based on DNA content" which requires "selecting by said cell sorting means the sperm having a DNA content corresponding to a desired chromosome which will produce a desired gender of offspring, and separating the selected sperm from nonselected sperm." Johnson-1992, claim 1. Johnson-1992 thus describes a flow cytometry-based process for sorting sperm that involves "identifying X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in the stream."

4. "collecting X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in at least one collector having a collector fluid that includes a citric acid"

Johnson-1992 describes collecting sperm cells containing either an X- or Y-chromosome into collection vessels containing a TEST egg yolk extender. Johnson-1992, 6:27-65; Pace-Decl. ¶ 214. Claim 1 of Johnson-1992 is directed to "[a] method for sorting intact, viable, mammalian sperm into X- and Y-chromosome-bearing populations based on DNA content" and "collecting the selected sperm in a viability-supporting collecting fluid." Johnson-1992, claim 1. Johnson-1992 thus teaches, "collecting X-chromosome bearing sperm cells and/or Y-chromosome bearing sperm cells in at least one collector having a collector fluid."

Johnson-1992 explains that sorting sperm into a collection container risks exposing them to stress caused by dilution, and then proposes options for mitigating that stress that include a collection fluid containing a buffered egg yolk extender. Johnson-1992, 4:51-58. Johnson-1992 also explains the collection fluid should be selected to maximize the viability of the sperm cells. Pace-Decl. ¶¶ 215-216. Johnson-1992, however, does not explicitly describe use of a collection fluid that includes a citric acid.

5. "fertilizing at least one egg with the collected sperm cells to form at least one sexed embryo"

Johnson-1992 discloses the successful insemination of mature New Zealand White does with sex-sorted sperm and birth of rabbit offspring. Johnson-1992, 7:21-33; Table II. Johnson-1992 also explains that using similar procedures, they achieved birth of piglets using sorted pig sperm. *Id.*, 7:36-41. Johnson-1992 thus discloses fertilizing at least one egg with the collected sperm by virtue of the production of offspring from does inseminated with sexed sperm. Pace-Decl. ¶ 219. As such, Johnson-1992 describes "*fertilizing at least one egg with the collected sperm cells to form at least one sexed embryo*."

D. Claim 11 Would Have Been Obvious to the Ordinary Artisan Based on Johnson-1992 in view of Salisbury-1978 and Further in View of Garcia-1989-III

As explained in § VI.C. Johnson-1992 describes methods that meet every limitation of claim 11, except that the sheath and collection fluids "*include a citric acid*." Modifying the Johnson-1992 process to use sheath and collection fluids that include a citric acid, however, would have been obvious to an ordinary artisan before the 1997 when applying its Johnson technique to sort bovine sperm in view of Salisbury-1978 and Garcia-1989-III. Pace-Decl. ¶ 221.

Initially, the ordinary artisan would have been motivated to use the Johnson-1992 method to sort commercially important sperm, such as bovine sperm, and that doing so would be a logical extension of the initial experimental examples in

Johnson-1992. Pace-Decl. ¶ 222. Sex sorting bovine sperm would be seen as providing significant advantages in breeding of cattle, such as to favor production of cows rather than bulls.

The ordinary artisan also would have understood from Johnson-1992 that sheath and collection fluids should be selected to maximize viability of bovine sperm, given the guidance in Johnson-1992 to do so, and that using consistent buffer systems known to be effective in maintaining the viability of bovine sperm would achieve this goal. Johnson-1992, 2:47-58, 4:23-6; Pace-Decl. ¶¶ 223-224.

That person also would have known that extenders and buffer solutions containing a citric acid maximize the viability of bovine sperm based on the teachings in Salibury-1978 and Garcia-1989-III and their extensive experience using such agents during the collection, processing and use of bovine sperm in conventional artificial insemination. Salisbury-1978, 442-443, 448; Garcia-1989-III, 1042, 1046; Pace-Decl. ¶ 225.

The ordinary artisan also would have expected use of sheath and collection fluids containing a citric acid to be as or more effective in obtaining viable bovine sperm as the exemplary conditions described in Johnson-1992. Pace-Decl. at ¶ 226. That person would have reasonably expected to achieve successful pregnancies when using the sorted bovine sperm using sheath and collection fluids containing a citric acid. Pace-Decl. at ¶ 226.

Consequently, claim 11 would have been obvious to the ordinary artisan based on Johnson-1992 in view of Salisbury-1978 and Garcia-1989-III.

1. An Ordinary Artisan Would Have Considered Johnson-1992 with Salisbury-1978 and Garcia-1989-III

The field of the '822 Patent is artificial insemination, a very well-established field before 1997. Buffers and extenders that had proven effective in maximizing the viability of different types of sperm during its collection, preparation and use were well known and extensively reported in the literature before 1997. *See* § IV.B. An ordinary artisan considering the application of the Johnson-1992 technique to sort commercially significant livestock, such as beef and dairy cows, would naturally have consulted this extensive literature to identify buffers and extenders that would maximize viability of bovine sperm. Pace-Decl. ¶ 228. Doing that would have revealed to the ordinary artisan suitable options for buffered solutions and extenders that would "maintain[] the viability of" bovine sperm as Johnson-1992 indicates is important for sheath and collection fluids. Johnson-1992, 2:47-58; *see also id.*, 4:19-22 ("It is, of course, of critical importance to maintain high viability of the intact sperm during the sorting process and during storage after sorting but prior to insemination.").

More specifically, based on Johnson-1992 and their experience, the ordinary artisan would have consulted well-known textbooks and publications for guidance on: (i) egg yolk extenders suitable for bovine sperm to use in the collection fluid to

offset the potential effects of dilution on the bovine sperm (*id*., 4:51-98, 6:40-7), and (ii) buffered solutions to use as sheath fluid that would meet the features identified by Johnson-1992 for bovine sperm. *Id*., 4:44-50, 6:38-40; Pace-Decl. ¶ 230. One such textbook is Salisbury-1978 – a leading authority on techniques for isolating, preparing and conditioning bovine sperm for artificial insemination. Pace-Decl. ¶ 231. Likewise, Garcia-1989-III provides extensive information on the effects of different buffered solutions on the viability of bovine sperm. Pace-Decl. ¶ 232. Both Salisbury-1978 and Garcia-1989-III also are plainly within the same field as the Johnson-1992 patent. Pace-Decl. ¶ 233. Consequently, an ordinary artisan would have considered the combined teachings of three references.

2. Salisbury-1978 Would Have Made Obvious Use of a Collection Fluid that Includes a Citric Acid When Sorting Bovine Sperm

Salisbury-1978 is one of the most respected texts in the field of artificial insemination of cattle. It provides a detailed description of the state of the art as of the time it was published. Pace-Decl. ¶ 139. Salisbury-1978 is the second edition of a textbook published in 1978, and has a Library of Congress Control Number 77013598 and ISBN of 0716700255. Further demonstrating its public availability before 1997, Salisbury-1978 was cited in many research articles in regularly published scientific journals published before 1997, including:

- Amann-1993 (Ex. 1023) at 405-406, published in 1993 in the Journal of Andrology;
- Odde-1980 (Ex. 1024) at 109, published in Theriogenology in 1980;
- Parrish-1986 (Ex. 1025) at 257, published in 1986 in Biology of Reproduction; and
- Stalhammar-1994 (Ex. 1026) at 44, published in 1994 in Reproduction Nutrition Development.

Salisbury-1978 is prior art to claim 11 of the '822 Patent under § 102(b).

a. Relevant Teachings of Salisbury-1978

Salisbury-1978 generally describes the physiology of bovine sperm cells and techniques used in artificial insemination of cattle, including the collection, evaluation, extension, and storage of bovine semen for subsequent artificial insemination. Pace-Decl. ¶ 140. Salisbury-1978 includes an in-depth discussion of bull semen extenders that are used to preserve sperm fertility and increase sample volume so that a proper dose of viable sperm can be conveniently packaged and used for artificial insemination. Salisbury-1978, 442-79.

Salisbury-1978 teaches that while there are "countless recipes for media that will preserve bull spermatozoa," the "yolk-citrate has been the standard against which new extenders have been compared." Salisbury-1978, 442, 448. It also points out that "[t]he yolk citrate extender, with various modifications, has been

the most widely used medium for artificial insemination in cattle." *Id*., 442-3, 448. Salisbury also explains yolk-citrate extenders provided superior results to extenders based on phosphates, stating:

> A major breakthrough in semen extenders came when Phillips (3) reported the value of hen's egg yolk. In the original formula the extender contained equal volumes of egg yolk and phosphate buffer. Salisbury et al. (4) showed that, by ***replacing the phosphate with sodium citrate***, equal fertility, higher spermatozoan motility during prolonged storage, and improved visibility resulted.

Id., 443 (emphasis added). Salisbury-1978 thus confirms that ordinary artisans working in the field of processing and handling bull semen before 1997 were aware of the advantages of using an extender containing a citric acid instead of a phosphate when working with bovine sperm.[5] Pace-Decl. ¶¶ 141-143.

Salisbury-1978 identifies three extender formulations that include a citric acid in conjunction with egg yolk which had been shown before 1997 to provide superior results on extending viability of bull sperm cells:

(i) Cornell University extender (CUE) (Salisbury-1978, 450, 452);

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[5] The inventor acknowledged this at his deposition. Seidel-Tr. at 39:11-40:10, 68:19-69:17, 70:19-25, 72:6-73:12, 73:16-74:7.

(ii) TRIS-egg-yolk-citric acid (Salisbury-1978, 450, 456), and

(iii) Egg-yolk citrate (Salisbury-1978, 447-8, 450).

See also CSS-1997, 6-7. The '822 Patent indicates that the CUE extender was suitable for use as a collection fluid. '822 Patent, 13:40-50 ("The Cornell Universal Extender may have the following composition for 1000 ml: 14.5 g sodium citrate dihydrate…0.87% citric acid…."). *See also* '822 Patent, Example 1 (specifying the use of CUE as the collection fluid).

Salisbury-1978 explains the "Cornell Universal Extender" (CUE) is made by adding both sodium citrate and citric acid with egg yolk to saline solution. Salisbury-1978, 450-3. It also explains the modified Tris based CUE extender is made using citric acid and egg yolk. Salisbury-1978, 457 ("The formula for preparing one liter of the CUE-Tris extender is…***5.53 grams citric acid,*** 10.88 grams sodium citrate….It is clear that Tris types of organic buffers….are suitable for ambient temperature storage, refrigerated, or frozen semen."). Salisbury-1978 explains that the egg-yolk citrate extender is formulated by combining sodium citrate dihydrate with egg yolk. Salisbury-1978, 447; Pace-Decl. ¶ 147. One commonality in these extenders (as well as others) is the presence of egg yolk, which was long recognized to be highly protective of sperm cells. Pace-Decl. ¶ 147.

The composition of these three widely used egg yolk-citric acid extenders is summarized below in Table 1.

Table 1. Most Commonly Used Bovine Extenders

Bovine Extender	**Components**	**Reference**
CUE	sodium citrate dihydrate 1.45g/100ml citric acid monohydrate 0.09g/100ml sodium bicarbonate 0.21g/100ml potassium chloride 0.0 g/100ml glucose 0.3g/100ml glycine 0.94g/100ml sulfanilamide 0.30g/ml 20 parts egg yolk	Salisbury-1978, 452.
Tris-egg yolk-citric acid	tris 3.028g citric acid monohydrate 1.675g fructose 1.25g egg-yolk 25ml glycerol 8.0ml Glass redistilled water 92.0ml	Salisbury-1978, 456.
Egg yolk-citrate	sodium citrate dihydrate 2.9% (w/v) egg yolk 20-25% (v/v)	Salisbury-1978, 447-448.

b. Using Well-Known Citric Acid-Egg Yolk Extenders in the Collection Fluid of the Johnson-1992 System Would Have Been Obvious When Sorting Bovine Sperm

Johnson-1992 makes clear that one should use a collection fluid in its sorting method that maintains "the viability of sorted sperm cells" (Johnson-1992, 2:56-58) and notes that "[i]t is, of course, of critical importance to maintain high viability of the intact sperm during the sorting process and during storage after sorting but prior to insemination." Johnson-1992, 4:19-22. The ordinary artisan

would have followed the guidance in Johnson-1992 and selected an extender solution that would maintain the viability of bovine sperm when using the Johnson technique to sort bovine sperm. Pace-Decl. ¶¶ 234-237.

The ordinary artisan would have known that extender solutions can have different effects on different species of sperm, and that certain extenders were more effective in extending the viability of particular species of sperm. Pace-Decl. ¶ 235. That was true for bovine sperm, one of the most commercially important and extensively studied types of sperm used in artificial insemination. Pace-Decl. ¶ 235. The ordinary artisan also would have understood the importance of selecting an extender suitable for bull semen – it was well known that even with the advances in the speed of the flow cytometers, samples take several hours to be processed and once sorted, the cells remain in the collection fluid, heavily diluted by the sheath fluid, for many hours longer. Pace Dec. ¶ 238.

Salibury-1978 teaches that the CUE, Tris-egg yolk-citric acid and egg yolk-citrate extenders were the most effective and proven extenders being used in processing of bovine sperm; each would have been recognized by ordinary artisans based on their experiences as meeting the criteria of an extender that would maximize bovine sperm viability as specified in Johnson-1992. *See* Salisbury-1978, 447-8, 452, 456; Pace-Decl. ¶¶ 241-242. The ordinary artisan also would have known before 1997 that citrate and citric acid-based extenders provide better

results than phosphate-based extenders for bovine sperm (Salisbury-1978, 443; Garcia-1989-III, 1039-40) and were known to keep bovine sperm healthy (Seidel-Tr., 39:11-40:10, 68:19-69:17, 70:19-25, 72:6-73:12, 73:16-74:7).

Consequently, a person of ordinary skill in the art considering how to adapt Johnson-1992 to sort bovine sperm would have found it obvious from Salisbury-1978 to use one of the well-known bovine semen extenders that contain a citric acid in the collection fluid when sex-sorting bovine sperm, such as CUE, Tris-egg-yolk-citric acid or egg yolk-citrate. Pace-Decl. ¶¶ 239-241.

3. It Would Have Been Obvious to Use a Sheath Fluid Including a Citric Acid Based on Johnson-1992, Salisbury-1978 and Garcia-1989-III

Garcia-1989-III was published in May of 1989 in the fifth issue of volume 31 of the regularly published scientific periodical, *Theriogenology*. It is the third of a series of papers published by Garcia and Graham that investigated various buffer systems for their effects on viability of bovine sperm. Pace-Decl. at ¶ 150.

Theriogenology is a well-known publication in the field of animal husbandry. For example, the '822 Patent incorporates by reference an article published in this journal. *See* '822 Patent, 12:34-8 ("… as published in 48 ***Theriogenology*** 1255 (1997) ***hereby incorporated by reference***.") (emphasis added). It would be implausible at best for Patent Owner to contend an article published in one issue of *Theriogenology* was sufficiently publicly available to

justify incorporating it by reference into its patent disclosure, but an article in a different issue of the same publication was not publicly available. Garcia-1989-III is prior art under §102(b) to the '822 Patent.

a. Garcia-1989-III Shows that Buffer Systems Including Citric Acid Perform As Well or Better than Those Including Phosphates in Maintaining Bovine Sperm Viability

Garcia-1989-III reports on experiments designed to investigate the effects of different acid-based buffer systems on the viability of bovine sperm. Garcia-1989-III, 1039, Abstract ("Three experiments were conducted to study the effect of inorganic and organic acids on survival of dialyzed bovine spermatozoa."). Among other things, Garcia-1989-III compared the effects of citrate and phosphate components of buffer systems on the viability of both fresh and previously frozen bovine sperm. *See* Garcia-1989-III 1039 (Abstract), 1040-1, 1042-43 (Table 1); 1046-47. Results of testing citrate buffers against phosphate and other buffers for effects on bovine sperm viability are reported in Table 1. From their results, Garcia-1989-III reports that sodium citrate-based buffers performed as well or better than phosphate-based buffers in promoting survival of the fresh and frozen-thawed semen, with some differences being more pronounced after isolation and counting of the cells via a Sephadex column. Garcia-1989-III, 1039, 1042, 1046; Pace-Decl. ¶¶ 153-154.

Table 2 reports results of testing citrate against other organic acids including tartrates, oxalates, succinates, formats and acetates. From that table, Garcia-1989-III reports:

> Results of the second experiment indicated that citrate, tartrate and oxalate salts provided superior ($P<0.05$) protection to the cells than salts of succinate, acetate or formate.

Garcia-1989-III, 1039 (abstract); 1042-4 ("Experiment 2" and Table 2).

Garcia-1989-III also reports that:

> The results of our study illustrate that post thaw sperm motility in solutions containing 35% isosmotic Na citrate (V/V) in the extender and dialysate was statistically superior to any other buffer solution studied. Sperm motility of fresh and frozen thawed samples was high in potassium phosphate and was not different from motility in samples containing Na citrate. However, the evaluation of the number of cells that passed through the Sephadex column showed statistical differences between the use of phosphate containing dialysates and Na citrate. All data obtained for carbonate and chloride solutions were significantly lower ($P<0.05$) than the data obtained for phosphate buffers.

Garcia-1989-III, 1046.

In other words, depending on the state of the sperm (i.e., fresh or frozen/thawed), Garcia-1989-III showed that buffers including a citric acid performed as well as or better than buffers containing phosphates in preserving

viability of the sperm. *Id*., 1039 ("Inorganic salts of phosphates, carbonates or chloride provided significantly less protection to the cells than the control extenders with Na citrate ($p<0.05$)"); 1042 ("Citrates and tartrates provided greater ($P<0.05$) protection to the cells before and after freezing than any other material used"); 1043 (Table I)); 1046.

Garcia-1989-III also introduced their experimental findings by reviewing the history of extenders and buffer solutions used in preparing bovine sperm for use in artificial insemination. It noted, *inter alia*, that prior research demonstrated that phosphates can impair aspects of sperm that cause them to become less viable, and that led to a greater use of buffers based on citrates:

> The first short term extender was developed in 1933 and is known as the Milovanov extender. It consisted of sodium sulfate (Na_2SO_4), glucose and peptone. In 1939, Philips (1), and later Philips and Lardy (2), recommended the use of a diluent containing equal parts of phosphate and egg yolk. Later, Bishop and Salisbury (3) reported that the motility and oxygen uptake of bull spermatozoa is depressed by high concentrations of phosphate. White (4) indicated that phosphate inhibits the oxidation of lactic acid and suggested that in phosphate diluents the O_2 uptake is low, and large amounts of lactic acid accumulate**.** Salisbury et al. (5) developed a sodium citrate extender containing 50% egg yolk, and Kinney and Van de Mark (6) found the optimal Na citrate concentration to be in a range of 1.55 to 1.95% (W/V).

Garcia-1989-III, 1039-40.

b. Garcia-1989-III Identified Benefits of Buffers Including a Citric Acid in Analogous Experimental Conditions

Garcia-1989-III demonstrated protective effects of different buffer systems on bovine sperm in a dialysis setting. In Garcia-1989-III and in its related paper, Garcia-1989-I, the details of these dialysis experiments are explained. In them, a bovine semen sample is first diluted with a conventional extender solution containing egg yolk combined with one of the buffer systems being tested. Garcia-1989-III, 1040-1; Pace-Decl. ¶ 160. A sample of the extended semen is placed in a dialysis bag, which is then dialyzed at 1:50 ratio relative to a solution containing the same buffer system without egg yolk. Garcia-1989-III, 1039 (Abstract); Pace-Decl. ¶ 161. As dialysis proceeds, the extended semen sample becomes diluted: through osmosis, constituents of the semen move out of the dialysis bag while the buffered solution passes into the dialysis bag. Pace Dec. ¶¶ 161-162.

The dialysis experiments described in Garcia-1989-III thus evaluated the effects of different buffer systems on the viability of bovine semen in an experimental setting analogous to that which occurs when sheath fluid dilutes the collection fluid in the collection container in the Johnson technique. In both, a buffered solution not containing egg yolk dilutes a sample of sperm extended with a semen extender containing egg yolk. Pace Dec. ¶ 160-164.

An ordinary artisan thus would have considered the experimental results reported in Garcia-1989-III to be relevant to the decision of which buffered solutions should be used as the sheath fluid when sorting bovine sperm in the Johnson technique. As noted earlier, Johnson-1992 identifies the importance of choosing a sheath fluid that will maintain the viability of the sperm during the sorting process. The ordinary artisan would have recognized from Garcia-1989-III (and elsewhere) that buffered solutions including a citric acid were as good, or better, than buffered solutions containing phosphates in maintaining the viability of fresh and frozen-thawed sperm, including in experimental settings where the buffered solution is diluting a sample of bovine sperm extended with a conventional egg yolk extender. Salisbury-1978, 442-443, 448; Garcia-1989-III, 1042, 1046; Seidel-Tr., 39:11-40:10, 68:19-69:17, 70:19-25, 72:6-73:12, 73:16-74:7; Pace Dec. ¶¶ 164-165.

c. It Would Have Been Obvious to Use a Buffer Including a Citric Acid in the Sheath Fluid When Using an Extender Including Citric Acid in the Collection Fluid in the Johnson Technique

As explained in § IV.A.4., sheath fluid propels a sample through the flow cytometer but does not mix with the sample layer until the sheath and collection fluids combine in the collection unit. Shapiro-1995, 16; '822 Patent FH, 235. Thus, as Johnson-1992 explains, the sheath fluid can affect sperm viability when it dilutes the collection fluid containing the sperm. Johnson-1992, 4:51-8. Johnson-

1992 also recognized that such dilution was a risk and provided a solution – namely, include an appropriate extender in the collection fluid. *Id.*

As explained in § VI.D.2.b, an ordinary artisan adapting the Johnson technique to sort bovine sperm would have selected extenders suitable for bovine sperm, the most common of which include a citric acid. Thus, as explained in § VI.D.2.b, Johnson-1992 in view of Salisbury-1978 would have made it obvious to use one of three well-known citric acid containing extenders to sort bovine sperm by the Johnson technique.

Johnson-1992 also teaches that one should select both a collection fluid and a sheath fluid that will maintain the viability of the sorted sperm cells. Johnson-1992, 4:23-6, 4:44-58. Given that guidance, the ordinary artisan would have found it logical to not only select a sheath fluid that had been shown to maintain the viability of the type of sperm being sorted but also to use a sheath fluid that would be compatible with the constituents of the extender media being used in the collection fluid. Pace-Decl. ¶¶ 248-249; *see also* § VI.D.3.c.

This latter principle was understood well before 1997. For example, as Leary et al. explained:

> Since the sorted sample will be highly diluted in sheath fluid, make certain that the cells can tolerate the sheath fluid. While phosphate-buffered saline (PBS) (and many cells dislike PBS!) is commonly used for sheath fluid in cell sorters other fluids can be used provided

> that they provide proper ion charges for cell sorting and do not disturb the viscosity for fluidic requirements or refractive index for optical requirements.

Leary-1994 (Ex. 1021), 350.

Thus, although PBS was used as a sheath fluid in the rabbit and swine examples in Johnson-1992, it would have been recognized as not being the optimal buffer media to use for bovine sperm. Pace-Decl. ¶ 251; Garcia-1989-III, 1046. For this reason, it would have been obvious to an ordinary artisan adapting the Johnson-1992 technique to sort bovine sperm to select a buffer to use in the extender and sheath fluids that includes a citric acid, as it was known that such buffers would maintain the viability of bovine sperm, particularly in view of the indication in Johnson-1992 of the possibility of a negative impact on the bovine sperm when the sheath fluid dilutes the collection fluid in the collection unit.[6] Pace-Decl. ¶ 252; Seidel-Tr., 138:6-139:15.

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[6] An ordinary artisan would have known from Johnson-1992 that agents such as egg yolk were unnecessary in the sheath fluid because it does not interact with the bovine sperm until it collects in the collection unit, which already has a protective environment created by the presence of the extender in the collection fluid. Pace-Decl. ¶ 252.

The ordinary artisan also would have understood that buffered solutions based on citric acid showed equivalent or better results with respect their effects on bovine sperm than buffered solution based on phosphates. Pace-Decl. ¶ 253; Garcia-1989-III, 1039 (Abstract); 1040-4, 1046, Table 1, Table 2; Salisbury-1978, 442-443, 448; *see* §§ VI.D.3.a- VI.D.3.b. That would have provided an additional reason for the ordinary artisan to select a sheath fluid including a citric acid rather than using one based on a phosphate buffer. The ordinary artisan also would have found it logical to use the other components of industry standard extenders (e.g., Tris-citric acid or CUE) in both the collection and sheath fluids, because doing so would lessen any impact caused by dilution from the sheath fluid of the characteristics of the collection media and would thereby minimize dilution-related stress on the cells. Pace-Decl. ¶ 254. Buffer solutions including a citric acid thus would have been considered by the ordinary artisan to be a known and predictable equivalents to the PBS sheath fluid used in Johnson-1992 when adapting those examples to sort bovine sperm. Pace Decl. ¶ 254-255.

The ordinary artisan therefore would have found it obvious to use a buffer solution including a citric acid as the sheath fluid when using the Johnson technique to sort bovine sperm, particularly when the extender used in the collection fluid also included a citric acid as suggested by Salisbury-1978. Pace-Decl. ¶256, 259. In fact, the inventor had the same conclusion – he testified that,

in 1997, using a sheath fluid containing a citric acid would have been an obvious choice before 1997 if the extender used in the collection container included a citric acid. Seidel-Tr., 138:6-139:15. Using a buffer solution containing a citric acid in both the sheath and collection fluids would have been expected to minimize negative impacts on the bovine sperm in the collection container when the sheath fluid combines with the collection fluid, given the knowledge that buffers including a citric acid were effective in maintaining the viability of bovine sperm in analogous experimental settings. Pace-Decl. ¶ 255.

Claim 11 of the ’822 Patent therefore would have been obvious based on Johnson-1992 in view of Salisbury-1978, when considered further in view of Garcia-1989-III.

E. An Ordinary Artisan Would Have Reasonably Expected that Flow Cytometry Using Fluids Containing Citric Acid Would Yield Viable Sexed Semen for Producing Sexed Embryos

Techniques for sorting sperm by sex characteristic had advanced significantly by the end of 1997. As the ’822 Patent explains, numerous improvements had been made to the basic system developed by Johnson in the late 1980’s. ’822 Patent, 2:10-23. In addition, the field of artificial insemination, and preparation of sperm samples to use in such procedures, had developed over decades, thereby providing extensive insight into how one should handle and

process sperm to ensure its viability for use in artificial insemination. Pace-Decl. ¶¶ 260-261.

An ordinary artisan equipped with this extensive knowledge by the end of 1996 would have reasonably expected to succeed in sorting bovine sperm using the Johnson technique illustrated in Johnson-1992, modified by the guidance in Salisbury-1978 and Garcia-1989-III to use collection and sheath fluids that include a citric acid, and to use the sorted bovine sperm to successfully impregnate a cow. Pace-Decl. ¶ 262. The success achieved in successful embryo creation in the examples in Johnson-1992 demonstrate that sufficient quantities of sex-sorted sperm can be recovered using the techniques shown in Johnson-1992, 7:1-41 (Examples 2-4); Pace-Decl. ¶ 263. Using conditions proven to be favorable to handling bovine sperm would only increase expectations of obtaining adequate yields of viable sex-sorted bovine sperm. Pace-Decl. ¶ 263.

Importantly, claim 11 sets no requirements for the yield of viable sorted sperm, nor does it set any threshold frequency of success in forming embryos. An ordinary artisan would have had high confidence that the Johnson-1992 scheme modified as described above would yield at least some sexed sperm that would successfully impregnate a cow. Pace-Decl. ¶ 264. Thus, an ordinary artisan would have reasonably expected a method of sorting bovine sperm using the Johnson-1992 method modified to use citric-acid containing sheath and collection fluids, to

be successful in producing at least one fertilized bovine embryo using the sex-sorted bovine sperm. Pace-Decl. ¶ 265.

F. No Secondary Considerations Support Non-Obviousness

While it is not the burden of Petitioner to disprove the existence of secondary considerations, it is relevant to note that no secondary considerations that Patent Owner may advance – commercial success, unexpected results or the like – would have a nexus to claim 11 of the ’822 Patent. As explained above, that claim differs from Johnson-1992 method in a single respect – use of collection and sheath fluids that include a citric acid. Claim 11 does not exclude any other agent being present in those two solutions, nor does it set any thresholds – minimum or maximum – for the amount of a citric acid to include in either solution. In other words, there are no elements within Claim 11 that could be correlated to any particular unexpected result that Patent Owner may assert. Moreover, given the interrelated nature of the agents used in bovine sperm extenders and buffer solutions, Patent Owner will not be able to prove that any particular secondary consideration would be attributable solely to the presence of a citric acid in either or both solutions. And it was firmly established in the prior art that citric acids contribute to the benefits seen with extender solutions and buffer solutions on viability of bovine sperm. Associating a known attribute of citric acid based solutions to any secondary consideration cannot render claim 11 non-obvious.

VII. CONCLUSION

For the foregoing reasons, the Petitioner respectfully requests that *inter partes* review be instituted and that claim 11 of the ’822 Patent be cancelled.

Dated: June 8, 2018

Respectfully submitted,

/Jeffrey P. Kushan/
Jeffrey P. Kushan
Registration No. 43,401
Sidley Austin LLP
1501 K. Street N.W.
Washington, D.C. 2005
jkushan@sidley.com
(202) 736-8914
Attorney for Petitioner

CERTIFICATE OF COMPLIANCE

I hereby certify that this Petition complies with the type-volume limitations of 37 C.F.R. § 42.24, because it contains 13,817 words (as determined by the Microsoft Word word-processing system used to prepare the Petition), excluding the parts of the Petition exempted by 37 C.F.R. § 42.24.

Dated: June 8, 2018

Respectfully submitted,

/Jeffrey P. Kushan/
Jeffrey P. Kushan
Registration No. 43,401
Sidley Austin LLP
1501 K Street N.W.
Washington, D.C. 20005
jkushan@sidley.com
(202) 736-8914
Attorney for Petitioner

PETITION FOR INTER PARTES REVIEW

OF U.S. PATENT NO. 9,365,822

Attachment A:

Proof of Service of Petition

CERTIFICATE OF SERVICE

I hereby certify that on this 8th day of June, 2018, a copy of this PETITION FOR INTER PARTES REVIEW has been served by Federal Express on the following address for Patent Owner(s):

XY, LLC
Attn: Ryan Christensen
Attn: Hashim Rahman
22575 State Highway 6 South
Navasota, TX 77868

Kirt S. O'Neill
Daniel L. Moffett
Clayton N. Matheson
George Andrew Lever Rosbrook
AKIN GUMP STRAUSS HAUER & FELD LLP
300 Convent Street, Suite 1600
San Antonio, TX 78205-3732

L. Jeffrey Hubenak
Locke Lord LLP
100 Congress Ave., Suite 300
Austin, TX 78701

Compass Bank
1703 W. 5th Street, Suite 500
Austin, TX 78703

Dated: June 8, 2018

Respectfully submitted,
/Jeffrey P. Kushan/
Jeffrey P. Kushan
Registration No. 43,401
Sidley Austin LLP
1501 K. Street N.W.
Washington, D.C. 2005
jkushan@sidley.com
(202) 736-8914
Attorney for Petitioner

PETITION FOR INTER PARTES REVIEW

OF U.S. PATENT NO. 9,365,822

Attachment B:

List of Evidence and Exhibits Relied Upon in Petition

Exhibit No.	**Reference Name**
1001	U.S. Patent No. 9,365,822 ("'822 Patent")
1002	U.S. Patent No. 9,365,822 File History ("'822 Patent FH")
1003	Declaration of Marvin M. Pace ("Pace-Decl.")
1004	Curriculum Vitae of Marvin M. Pace
1005	U.S. Patent No. 5,135,759 ("Johnson-1992")
1006	Salisbury et al., Physiology of Reproduction and Artificial Insemination of Cattle, 2d ed. (1978) ("Salisbury-1978")
1007	Garcia et al., "Development of a buffer system for dialysis of bovine spermatozoa before freezing," Theriogenology, 31(5): 1039-1048 (1989) (Garcia-1989-III)
1008	Shapiro, Practical Flow Cytometry (3d ed. 1995) ("Shapiro-1995")
1009	Johnson et al., "Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting," Bio of Reprod, 41: 199-203 (1989) ("Johnson-1989")
1010	Johnson, "Sex Preselection in Swine: Altered Sex Ratios in Offspring following Surgical Insemination of Flow Sorted X- and Y-Bearing Sperm," Reprod Dom Anim, 26: 309-314 (1991) ("Johnson-1991")
1011	Cran et al., "The predetermination of embryonic sex using flow cytometrically separated X and Y spermatozoa," Human Reprod Update 2(4): 355-363 (1996) ("Cran-1996")
1012	Shapiro, Practical Flow Cytometry (4th ed. 2003) ("Shapiro-2003")
1013	Johnson, "Sex Preselection by Flow Cytometric Separation of X and Y Chromosome-bearing Sperm Based on DNA Difference: a Review," Reprod Fertil Dev, 7: 893-903 (1995) ("Johnson-1995")
1014	Seidel G.E., "Fertility of Bulls on the Edge of the Dose-Response Curve for Numbers of Sperm per Inseminate," 17th Technical Conference on Artificial Insemination and Reproduction, September 25-26 1998 ("Seidel-1998")
1015	Kling, "Flow Cytometry: It's Not Just For Immunologists Anymore," The Scientist, June 23 (1997) ("Kling-1997")
1016	"Minimum Requirements for Disease Control of Semen Produced for AI." ("CSS-1997")
1017	Masterton, Chemical Principles Using the International System of Units (4th ed. 1977) ("Masterson-1977")

Exhibit No.	Reference Name
1018	Graham et al., "Effect of some zwitter ion buffers on the freezing and storage of spermatozoa I. Bull," J. Dairy Sci. 55(3): 372-378 (1972) ("Graham-1972")
1019	Johnson et al., "Modification of a Laser-Based Flow Cytometer for High-Resolution DNA Analysis of Mammalian Spermatozoa," Cytometry, 7:268-273 (1986) ("Johnson-1986")
1020	Seidel, "Uterine horn insemination of Heifers with very low numbers of nonfrozen and sexed spermatozoa," Theriogenology, 48(8): 1255-1264 ("Seidel-1997")
1021	Leary, "Strategies for Rare Cell Detection and Isolation," Methods in Cell Biology: Flow Cytometry, 2nd Ed. Vol. 42 (1994) (Leary-1994)
1022	Johnson, "Isolation of X- and Y-bearing sperm for sex preselection," Oxford Reviews of Reproductive Biology, Vol. 16 (1994) ("Johnson-1994")
1023	Amann et al., "In vitro evaluation of sperm quality: an opinion," Journal of Andrology, 14(6):397-406 (1993) ("Amann-1993")
1024	Odde et al., "Short estrous cycles and associated serum progesterone levels in beef cows." Theriogenology, 14(2):105-112 (1980) ("Odde-1980")
1025	Parrish et al., ""Fertility of cooled and frozen rabbit sperm measured by competitive fertilization," Biology of Reproduction, 35:253-257 (1986) ("Parrish-1986")
1026	Stalhammar et al., "The impact of sperm motility on non-return rate in preselected dairy bulls," Reprod. Nutr. Dev. 34:37-45 (1994) (Stalhammar-1994)
1027	Garcia et al., "Development of a Buffer System for Dialysis of Bovine Spermatazoa before Freezing: I. Effect of Zwitterion Buffers," Theriogenology, 31(5): 1021-28 (1989) ("Garcia-1989-I")
1028	Garcia et al., "Dialysis of Bovine Semen and Its Effect on Fresh and Freeze-Thawed Spermatazoa," Cryobiology, 24:446-454 (1987) ("Garcia-1987")
1029	Seidel Deposition Transcript. May 8, 2018. ("Seidel-Tr.")