Smart Screening Increases Successful IVF Pregnancies

Researchers are harnessing the speed and accuracy of the VeriSeq™ PGS Solution on the MiSeq® System to assess the genetic viability of embryos.

Introduction

When Louise Brown, the first so-called "test tube" baby, was conceived at a Cambridge, England fertility clinic in 1978, the public reacted skeptically. In vitro fertilization (IVF), an assisted reproductive technology that fertilizes human eggs in a laboratory and transfers the embryos into a woman's uterus for implantation, was frightening to them. As more children were born through IVF, people grew to accept the procedure. IVF is now a proven method for overcoming infertility, changing the lives of millions of women and their families.

At its simplest, the process involves stimulating the ovaries to produce multiple eggs, collecting and fertilizing those eggs, selecting good quality embryos, and transferring them into the uterus for implantation. To achieve the best outcome—carrying a pregnancy through to delivery—it is important to identify the most viable embryo for implantation. Thanks in part to array screening and next-generation sequencing (NGS) technologies, the process has evolved over the years to allow doctors to examine and select the embryo that is the most viable genetically and therefore most capable of resulting in a successful pregnancy.

Genea is a leading company specializing in fertility and IVF treatment based in Sydney, Australia. It has spearheaded cutting-edge fertility treatments and technologies by emphasizing in-house research and development. iCommunity spoke with Don Leigh, PhD, Principal Scientist at Genea, about the company's use of Illumina NGS systems and array products for preimplantation genetic screening (PGS), and how it's lowering the cost for its customers.

Q: How did you become interested in molecular genetics?
Don Leigh (DL): I actually got into the field by accident. My background was originally in biotech, microbial genetics, and physiology. After completing my PhD, I shifted into molecular genetics, and then into cancer genetics. In the early 1990s, I studied the molecular diagnostics of muscle diseases in a large public hospital. That was where I first encountered people performing IVF. Genea, which was Sydney IVF at the time, wanted to initiate molecular embryo testing within the company. They recruited me in 1998 to set up a gene testing laboratory for embryo analysis.

Q: When was Genea originally founded and what did it offer?
DL: Genea was founded about 28 years ago as Sydney IVF. We performed prenatal diagnosis for 22 years and first trimester screening for at least 15 years. Our late founder, Professor Rob Jansen, changed the name to Genea 4 years ago to reflect the expanded focus of the company. He wanted our services to span the time before conception to the delivery of a child. We're no longer focused solely on IVF. We're moving heavily into molecular testing and setting up services that are complementary to IVF. We have units that offer massage therapy, health and exercise advice, and we even have personal trainers for our patients.

Q: What is your role at Genea?
DL: After developing the embryo testing capabilities at Genea, I became more involved in research about 12 years ago. That's when we started thinking about not just looking at the embryo, but looking at the patients—the woman, or the woman and her partner.

Q: How has IVF changed over the years?
DL: The IVF world is moving away from traditional IVF. With improvements in embryo freezing techniques, it's moving towards the concept of IVF as an egg collection/embryo creation exercise, followed by a pregnancy creation exercise. It enables couples to produce embryos and hold off starting a family for a few years or planning the next part of their family earlier. For older women, the techniques enable saving of multiple high-quality embryos.

There have also been changes in how IVF pregnancies are perceived. Our view is that a healthy IVF pregnancy results in 1 baby. This is a concept that Prof. Jansen worked on for 20 years. He began using advanced embryo culture, enabling selection of the best embryo to improve pregnancy rates.

Q: What new technologies has Genea developed?
DL: We're focused on developing new technologies and equipment that benefit the IVF field. One of the most common embryo transfer catheters used today was developed by Prof. Jansen. We developed the Genea Automated Vitrification Instrument (Gavi), the world's first automated vitrification (rapid freezing) device for storing eggs and embryos. It enables the automation and standardization of the embryo equilibration process to make it more consistent. We have also

Don Leigh, PhD, is a Principal Scientist at Genea Ltd.

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developed an embryo incubator system called Geri that incorporates a camera system in each chamber to take images of the developing embryo. We're using these technologies to broaden the services we offer to the industry and improve our implantation rate, with the ultimate goal of improving patient outcomes.

Q: How does Gavi improve egg and embryo freezing?
DL: Good freezing technique is the main thing that allows high-quality total chromosome screening to be viable on a wider scale. It maintains the embryo's viability, enabling it to be thawed successfully. While vitrification is highly effective, it's dependent on the skill of the technician. Poor freezing technique results in a nonviable embryo. Unfortunately, there's no way to know if vitrification has been performed correctly until the embryos are thawed. We decided to develop an instrument to standardize and automate the important equilibration process, enabling the process to be extremely consistent.

Q: What are the benefits of developing an embryo incubator with integrated camera system?
DL: The ability to take images of embryos without removing them from their optimal environment represents the next generation of individualized incubators. The Geri enables uninterrupted embryo incubation, with each incubator chamber having its own video capture system. It allows the embryo to divide and grow undisturbed and enables the assessment and tracking of embryo growth remotely. It provides additional viability information to complement PGS.

> PGS benefits patients by enabling us to identify the most viable embryos, increasing the chances of a successful pregnancy in the first attempts.

Q: How have the company's research interests evolved over the years?
DL: Prof. Jansen always said that he was a clinician who was really a frustrated scientist. He supported an active research program at Genea. About 12 years ago, we started building our own microarrays, but we found that it didn't make commercial sense. We then turned our focus to producing embryonic stem cells from our PGD (preimplantation genetic diagnosis) program. The embryos have specific monogenic diseases, so we know that they contain genes related to Huntington's disease, muscular dystrophy, etc. We develop, culture, and bank them for distribution to research groups studying certain diseases. We're the first group to generate stem cells under GMP (good manufacturing practice) conditions. The stem cell group will soon set up operations in La Jolla, California as Genea Biocells.

Q: Why are you moving from array platforms to sequencing platforms for PGS?
DL: We're moving to the MiSeq System as our PGS sequencing platform to meet quality and workflow demands. We have used 24sure® microarrays for at least 3 years. The 24sure microarray is cost effective and we use it to identify aneuploidy (chromosomal abnormalities) and certain types of translocations (chromosomal rearrangements). We found it had its advantages and limitations.

Sequencing results are comparable with microarrays, but sequencing data are more comprehensive and higher in quality and resolution. Microarrays rely on external controls that can influence the quality of the reads. Any disturbance in those controls affects every sample. With its internal controls, sequencing doesn't depend on an externally derived reference point. Instead, it looks at the amplified sample directly to compare chromosomes, thus reducing noise.

> The MiSeq System itself is very simple to use and it's the foundation of a VeriSeq PGS workflow process that is much easier than other technologies.

Q: Why did you switch from the Ion Personal Genome Machine to the MiSeq System to perform NGS-based PGS?
DL: We started looking at the MiSeq System because it was cost-effective and had a workflow that could be automated. We also wanted to increase access to genetic screening for our patients; NGS was the obvious way to go. Our previous financial model for PGS had everyone paying the same price, whether we biopsied 1 or 10 embryos. We've transitioned to a lower per embryo charge and now we have an average of 30–40% of IVF cycles being screened. We have some days when it's 80%.

We're looking at how to make the service even more accessible and how to handle it logistically. We feel the MiSeq System will enable us to streamline the workflow. We just installed it to perform PGS and are in the validation process. Everything is working fine.

Q: How do you find using the MiSeq System?
DL: The MiSeq System itself is very simple to use and it's the foundation of a VeriSeq PGS workflow process that is much easier than other technologies. We intend to streamline the workflow even further by automating library preparation.

Q: How will you decide when to use 24sure microarrays and when to perform VeriSeq PGS with the MiSeq System?
DL: We want to move as much as we can to VeriSeq PGS after the MiSeq System validation phase is completed. We already have several liquid handler robot systems that we are programming to automate some of the MiSeq library preparation steps.

Q: Will you be using the BlueFuse® Multi Software to analyze the results?
DL: Our staff has been using BlueFuse software for a while now and it's great. We're happy that we'll continue benefiting from software that they're familiar with and comfortable using.

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Q: How has NGS-based PGS testing impacted your IVF center and benefited patients?
DL: NGS allows us to handle the current PGS testing requirements and prepare for future demand. We're a fairly large group and we do about 5000 egg pickups a year. We're in the planning stages of how we can deal with a growing number of testing applications. Internally, we're doing between 80 and 100 embryos a week now, and it's going up.

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Q: What technologies do you think will have the biggest impact on the IVF field in general?
DL: Anything that lets us ascertain the quality of the embryo will have a large impact. It was only 5–6 years ago that PGS was demonstrated and shown to be feasible technologically, even though an important aspect, blastocyst stage biopsy, was first demonstrated by our group 16 years ago. The capability of looking at more chromosomes now let us see the true impact and benefit of checking each embryo for chromosomal content. Arrays made it possible and vitrification made it useful, enabling us to freeze and store genetically viable embryos. Chromosome testing will have a large impact for years to come.

Many of these things are contingent upon people actually practicing good IVF in the first place. Then we'll see the full benefit of combining chromosome testing with a freeze-all embryo approach that will enable patients to plan their pregnancies for the next 2–6 years.

Learn more about the Illumina products and systems mentioned in this article:

- MiSeq System, www.illumina.com/systems/miseq.html
- NextSeq 500 System, www.illumina.com/systems/nextseq-sequencer.html
- VeriSeq PGS Solution, www.illumina.com/products/veriseq-pgs.html
- BlueFuse Multi Software, www.illumina.com/clinical/clinical_informatics/bluefuse.html
- 24sure microarray, www.illumina.com/products/24sure-microarrays.html

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