Biomarkers in reproductive medicine: the quest for new answers

Carlos Simon\textsuperscript{1,2,*}, Denny Sakkas\textsuperscript{3}, David K. Gardner\textsuperscript{4}, and Hilary O.D. Critchley\textsuperscript{5}

\textsuperscript{1}Igenomix, Parc Cientific Universitat de Valencia, Valencia, Spain \textsuperscript{2}Department of Obstetrics and Gynecology, University of Valencia, INCLIVA Health Research Institute, Fundació IVI, Valencia, Spain \textsuperscript{3}Boston IVF, Waltham, MA, USA \textsuperscript{4}School of BioSciences, University of Melbourne, Parkville, VIC, Australia \textsuperscript{5}MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

*Correspondence address. E-mail: carlos.simon@ivi.es

Personalized medicine in assisted reproductive technologies (ART) is still in its infancy. Precision medicine, based on objective molecular tools added, to clinical medicine is well-accepted and developed in oncology and other potent medical disciplines. The impact of personalized medicine in cancer is broad, from screening to diagnosis, with the stratification of patients into cancer subgroup categories, facilitating individualized therapies that impact treatment effectiveness and disease recurrence (Diamandis \textit{et al.}, 2010; Hamburg and Collins, 2010).

In reproductive medicine many ‘unknown black boxes’ still exist. These will only be unraveled with the timely application of novel technologies and knowledge from modern medicine, complementing classical clinical protocols. Albert Einstein wrote: ‘If you want different results, do not do the same’. This quotation is applicable to the current status of our field. In addition to classic single molecular markers, high-throughput approaches may be used for personalized diagnosis, including next generation sequencing (NGS), single-nucleotide polymorphisms (SNP), microarray analysis or mass spectrometry. A common trend among these tools is their ability to analyze a myriad of targets simultaneously. Consequently with the addition of bioinformatics and systems biology to the equation, the sensitivity, specificity, and accuracy, as well as the complexity of new biomarkers discovered, is increasing.

The complication of applying personalized medicine in reproduction is that it is not ‘personalized’ to one individual but in fact three; the mother, father and offspring. A further complication is that we are trying to achieve a personalization of different biological systems; the egg, sperm, embryo and uterus. All these entities carry with them a predefined contribution to our final goal of achieving a healthy live birth. This contribution is in some cases greater or lesser from any one of the four biological systems but regardless of the level it will always impact on the final reproductive outcome.

Understanding and deciphering the individual and combined contribution of these systems was the main theme of the 2nd Reproductive Biomarker Meeting held in Valencia (Spain) in April 2014. This conference was attended by \textgreater{} 300 international delegates who revisited fundamental concepts in the light of new scientific knowledge as applied to improve diagnosis and thereby treatment effectiveness in our discipline.

In this issue of Human Reproduction Update perspectives from the oocyte (Iliodromiti \textit{et al.}, 2015), sperm (Sakkas \textit{et al.}, 2015) and embryo (Gardner \textit{et al.}, 2015) are presented.

In the ovarian stimulation field, significant progress has been made toward the translational application of direct (bio) markers, such as anti-Müllerian hormone (AMH), and antral follicle count (AFC). The current evidence base contrasts with what was previously considered ‘state of the art’ indirect (bio) markers, such as FSH, maternal age, or complicated ovarian-response prediction tests (Iliodromiti \textit{et al.}, 2015). AMH and AFC reference values for both biomarkers have changed substantially, and further change is expected. Both reflect a very similar ovarian follicle population that, if perfectly measured, would be expected to have similar values that complement each other. Iliodromiti and colleagues propose and demonstrate that AFC and AMH have complementary roles in the pre-assessment of the ovarian reserve that is now applied in infertile woman, but will soon be extended to screen women personalizing their reproductive potential.

Although obtaining the best oocyte from an effectively stimulated ovary is a first thought of the reproductive clinician it is also undeniable that the sperm will impact the likelihood of success of reproduction. Indeed we are now discovering that the paternal effect impacts not only embryo quality but can also affect early pregnancy loss/miscarriages, late onset diseases and also future generations. The advent of IVF and ICSI has made the analysis of more than just sperm numbers and motility important as many cases treated by ICSI utilize spermatozoa that would never have the ability to reach the egg in a natural environment.

We thus need to search for more subtle sperm biomarkers if we aim to understand how a sperm can impact outcomes. Sakkas and colleagues argue that although some of the current sperm selection techniques are providing some improvement we need to return to see how nature deals with sperm to improve our own ART outcomes (Sakkas \textit{et al.}, 2015). Of the millions of sperm ejaculated it is only a small number that arrive to the egg. Sakkas and colleagues argue that the natural sperm selection processes can be used to identify biomarkers of the best sperm qualities and mimic the same processes to select them.

Sakkas and colleagues also examine how new massive molecular analysis techniques [the omics] may be used to tie in transcriptomics, proteomics and/or metabolomics to male fertility. More importantly they
evaluate how our greatest challenge is creating predictive tests or models that can incorporate the multifactorial nature of fertility. Sakkas and colleagues therefore present the thesis that identifying the attributes of the spermatozoa that are able to successfully navigate the female reproductive tract and arrive at the site of fertilization will allow us to also identify the cells that possess all, or the majority, of the best traits for selection in ART. After all these cells are the ones that are able to surpass all the natural challenges put in place over millions of years of evolution. Understanding their characteristics (DNA/chromatin, membrane integrity, morphological traits and ‘omics’ profiles) will allow us to develop functionally relevant diagnostic and sperm selection strategies to maximize reproductive success (Sakkas et al., 2015).

Historically, the ART community’s response to an inability to select the best gametes and embryo, ensuring a higher chance of a live birth, was to transfer multiple embryos back to patients. Transfer of more than one embryo in an IVF cycle creates the dangerous possibility of a multiple gestation. Even a twin pregnancy bestows risks to mother and babies alike. Therefore, the move to single embryo transfer for all patients will be expedited by the ability to quantify viability. For nearly 40 years, morphology (assessed at a few discrete time intervals) has been the sole means available to the clinical embryologist to gauge the development of the human embryo in utero. Although several elegant grading systems have been developed to assist in the application of such information to help in embryo selection for transfer, the analysis of morphology alone cannot account for the physiology or karyotype of the embryo. With the advent of commercially available, reliable and effective time-lapse microscopy systems, we are now in a position to assess the embryo at almost unlimited time points. Consequently we are learning more about the impact of specific cleavage patterns, such as direct cleavage to the 3-cell stage (which reflects a compromised and/or aneuploid embryo), and the significance of timings associated with key developmental events.

As discussed by Gardner and colleagues, a key application of such data is the ongoing development of algorithms to assist in embryo deselection (Gardner et al., 2015). Together with the analysis of embryo metabolism, which not only appears to be associated with embryo viability but has recently been shown to be related to morphokinetic data, we are entering an era where the quantification of viability prior to transfer is looking more promising than ever. Concurrently, we have witnessed rapid developments in molecular techniques to accurately determine the chromosomal complement of the embryo, with a growing move towards trophectoderm biopsy at the blastocyst stage. Hence, we are now in a much stronger position to identity euploid embryos prior to transfer, thereby not only increasing implantation and pregnancy rates but also significantly reducing the time to pregnancy for patients, decreasing patient dropout rates from their clinical treatment. Looking to the future it is envisaged that embryos will have their development monitored through time-lapse, the surrounding medium analyzed and the physiology of the embryo quantitated, and where advocated their trophectoderm biopsied (together with vitrification of the blastocyst). The latter will facilitate transfer of the most viable euploid embryo in a subsequent natural cycle. Microfluidic devices, integrated into time-lapse systems, may well represent the platform through which this becomes a practical reality for the IVF laboratory (Gardner et al., 2015).

The 2nd Reproductive Biomarker Meeting was not limited to discussion and debate solely on gametes and embryos but endeavored to embrace how novel and emerging biomarker discovery might impact on reproduction beyond implantation successes or failure. There is now a spectrum of existing and rapidly developing technologies such as deep-sequencing, video-lapse, genomics, transcriptomics, bioinformatics, proteomics and metabolomics all with the potential to further the identification of new biomarkers across the wider field of reproductive health.

It is hoped that as biomarkers emerge we will achieve a better understanding of the myriad of complex reproductive processes that if not finely tuned result in early pregnancy failure, pregnancy complications and even later-life consequences. We need not only to be better able to predict ovarian reserve, endometrial receptivity, gamete quality, embryo viability and euploidy. We also need to be able to identify biomarkers that will confidently diagnose ectopic pregnancy, pre-eclampsia and preterm labor, at their earliest stages, thereby facilitating appropriate prophylaxis and/or better targeted treatment.

Examples of personalized diagnostics in reproduction are now being adopted clinically. A molecular diagnostic test is clinically available to personalize the endometrial receptivity of individuals.

The endometrial receptivity array (ERA) can determine the endometrial receptivity status of a patient by the identification of the transcriptomic signature composed by the expression of 238 genes. The profile differences are implemented in a computational predictor that can diagnose objectively the personalized window of implantation (WOI) of a patient (Diaz-Gimeno et al., 2011). We have always assumed that the endometrial WOI is always there whenever the embryo is ready to be transferred. The importance of the ERA test is that it challenges the thought that the WOI is constant in all patients. By identifying WOI displacements in patients with recurrent implantation failure it introduces the concept of personalized embryo transfer to treat a previously unknown endometrial pathology that may be the result of individual physiological variation (Ruiz-Alonso et al., 2013).

A further example of an emerging biomarker technology is the non-invasive prenatal diagnosis (NIPT) of fetal DNA in maternal blood identifying chromosomes 21, 18, 13, X and Y as early as the 10th week of pregnancy, and now clinically available worldwide (Bianchi, 2014).

We must also not forget that many benign gynecological complaints may have the potential to impact on future fertility and reproductive success. Common conditions, each with a major impact on quality of life, such as endometriosis and uterine fibroids (leiomyomas) may contribute to infertility and poor obstetric outcomes. Both these conditions are more common as women become older. The quest for diagnostic and therapeutic biomarkers for these conditions is thus no less important if an improved reproductive outcome is our desire. This is especially pertinent as we now progress through the 21st century and when in many regions of the world couples are delaying family and thus need to both retain and optimize their fertility potential.

Surely it is across the spectrum of reproductive health, of both men and women, that we have identified and discussed herein that the new biomarker technologies will have a crucial role to play.

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References


