Improving IVF outcomes: Development of a serum-based assay to predict endometrial receptivity in the days before embryo transfer.

An estimated 121 million couples worldwide suffer infertility, with around half of these seeking medical assistance to start a family. There is increasing demand for assisted reproductive technologies (ART), including in vitro fertilisation (IVF). Contrary to popular belief IVF, with its high emotional and financial costs, does not hold the answer for many women. Despite advances in embryo quality testing and selection, success rates worldwide are still below 30%. Up to 50% of women who undergo six embryo transfers will remain childless, with these repeat failures due to endometrial failure.

A recognised limitation of current clinical practice is our inability to effectively monitor a women's endometrium and its development into a 'receptive state' essential for successful embryo implantation, development of pregnancy and birth of a healthy baby.

Endometrial receptivity is a crucial factor determining whether IVF treatment will be successful or not, and current high failure rates are attributed to poor endometrial receptivity and synchrony. Embryo transfer needs to take place when the endometrium is receptive and capable of supporting a healthy pregnancy. Traditionally, it was thought that this timing – the 'window of implantation' – was the same for all women. However, for one in four women the timing of this receptive stage is different, and the traditional timing of embryo transfer does not work. Even more importantly, the hormonal treatments used for ovarian stimulation in an IVF cycle can dramatically alter the endometrium, resulting in non-receptivity in that cycle and hence failure of a transferred embryo to implant.

The ability to test endometrial receptivity in real-time, and determine whether or not a women's endometrium will be receptive to an embryo in that cycle, would allow clinicians and patients to know when embryo transfer could take place to maximise the chances of a successful pregnancy. If a test can show that a woman's endometrium is not receptive to an embryo (so pregnancy is not likely to result) the clinician could instead freeze the embryo for transfer in a later, better cycle. Such a test would reduce wastage of high-quality embryos and improve success rates.

However, there is no test available in the marketplace that can tell if the endometrium will be friendly or hostile to an arriving embryo just a few days later.

The ideal test for endometrial receptivity

Currently, endometrial receptivity can only be tested in the cycle before embryo transfer takes place – not in the same cycle as transfer will actually happen. The 'Endometrial Receptivity Array' (ERA) is an invasive genetic test requiring a sample of endometrial tissue, taken in the cycle prior to implantation. Furthermore, the ERA only tests movements in the timing of receptivity in the cycle tested, not whether it will be achieved in the cycle of transfer.

Our team has developed a non-invasive serum-based multivariate diagnostic algorithm that can predict endometrial receptivity in the days prior to embryo transfer, enabling decision making as to whether to transfer in the same cycle as the test is performed, or to freeze the embryo for a better opportunity.

Our current test consists of four biomarkers, tested in a Luminex™ multiplex assay. A further biomarker was then identified and also tested using Luminex™ technology. These two tests combined, along with known fertility factors of age and BMI, results in an algorithm that predicts endometrial receptivity and the likelihood of achieving a successful embryo implantation in a given cycle. It provides a real-time assessment of the endometrial quality, so clinicians and patients can make informed decisions about treatment.

The ideal test is:	Our assay	ERA assay – current standard
simple and non-invasive	Uses a serum sample, which can be easily collected in the clinic.	Uses endometrial tissue, which is invasive and damages the endometrium.
easy to perform	5 protein assay using standard Luminex™ technology.	236 gene array.
available	Can be done on-site at a local pathology provider or fertility centre.	Samples are submitted to a single laboratory, with special requirements for shipment.
fast	Results in 12 hours.	Results in 10 days from sample receipt.
compatible	Non-invasive sample collection, and minimal likelihood sample is incompatible with test.	<5% chance of insufficient sample for test, leading to additional cycles and sample collection.
personalised	Multiple tests can be done in a single cycle to effectively monitor each woman's own window of implantation.	Multiple testing takes multiple cycles to determine the window of implantation.
real-time	Our test represents the endometrium in the cycle of transfer, so no additional costly cycles are needed.	Must be done the cycle prior to embryo transfer and presumes every cycle will be same.

Development of an improved test

For development into a commercially viable product, the five biomarkers would ideally be assayed in a single test. This would reduce both the time and cost required to perform the assay. To reach this point, the combination of all five biomarkers needs to be assessed for cross-interference between analytes and assay performance characteristics. While studies to date have utilised Luminex™ technology, any platform capable of quantitative protein antigen measurement could potentially be used for further development of this assay.

The team aims to re-establish their predictive algorithm using the single assay format, checking its performance using the original 254 patient sample set against the current two-test assay format. The algorithm performance will then be further validated using serum samples collected from three independent IVF clinics, to ensure the assay can be applied in different geographical regions.

Funding request

We are seeking AUD 0.7 million investment over 2 years to complete development of the test ready for commercialisation. The funding includes staffing, laboratory consumables and intellectual property management costs.

Market

Infertility rates are increasing and currently 17% of couples in the developed world are seeking medical intervention for failure to conceive. There are over 1.2 million assisted reproductive therapy cycles performed annually, with only 20% resulting in a live birth. It is estimated that the test could generate between $100 m and $200 m per annum.

One of the major players in the global IVF and fertility technology space, Merck KGaA, have previously supported this research, including funding from their 'Grants for Fertility Innovation' program. This market leader remains a potential future industry partner for commercialisation and/or distribution of any market ready products arising from this development program.

Intellectual property position & ownership

The Development Plan includes a portion of costs for managing the existing IP and any new IP developed during the course of the project. Hudson has taken out, and is sole owner of, two patent applications:

1. WO/2015/149129 (priority date 2 April 2014) which is currently progressing in National Phase in USA, Europe, China, Canada and Australia.
2. WO/2017/054038 (priority date 30 Sept 2015) which is due to enter National Phase on 30 March 2018. This second application also includes a potential therapeutic option to be developed, focussing on one of the biomarkers in the assay.

Supporting data

An optimal multivariate signature, using logitboost, using Weka™ software to predict successful pregnancy was generated that combines age, serum progesterone, serum estradiol, and the five biomarkers (in the two-test format). Receiver-operator-curve (ROC) analysis was performed for the full cohort and for two sub-cohorts comprising day 3 and day 5 transfers. The generated ROC plots are shown below (Figure 1).

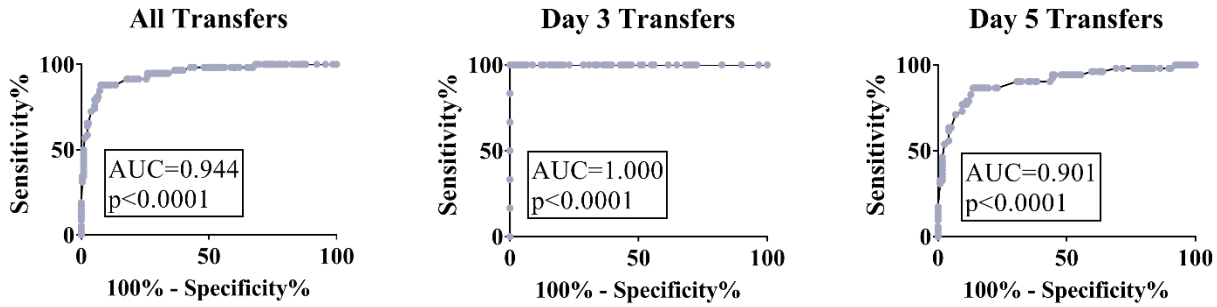

Figure 1. Receiver-operator-curve (ROC) analysis of the predictive algorithm performance of all transfers, day 3 and day 5 (C) cohorts.

Assay performance found 87% specificity at a sensitivity of 80%. Confounder analysis found BMI, patient etiology and prior cycles were not significant in the performance of the algorithm. The performance of the algorithm in discriminating outcome was greatest with high quality grade 1 and 2 embryos ($p<0.0001$), while less but still significant with grade 3 embryos ($p<0.05$).

Team

The Hudson team is an internationally recognized research group that has a long history of high level research in endometrial receptivity, complemented by expert clinicians with experience in fertility and assisted reproductive technologies.

Dr Tracey Edgell, PhD brings strong expertise in antibody and protein technologies, protein biomarker discovery and assay development, coupled with experience in regulatory body requirements and quality assurance. Prior to joining Hudson Institute, Dr Edgell spent more than a decade working in the Australian biotechnology sector as a R&D scientist.

Professor Lois Salamonsen PhD, FRANZCOG(Hon), FAA is an internationally-recognized leader in human uterine biology, fertility & infertility with a particular focus on endometrial receptivity. With a research career spanning more than 30 years, she is Head of the Endometrial Remodelling Research Group at Hudson Institute, a Fellow of the Australian Academy of Sciences and holds an Adjunct Professorship in the Department of Obstetrics and Gynaecology at Monash University.

Professor Luk Rombauts MD PhD FRANZCOG CREI is the National Medical Director of Monash IVF and has been Monash IVF's Clinical Research Director since 2001. He is also the Head of Reproductive Medicine at Monash Health, is an Adjunct Clinical Associate Professor in the Department of Obstetrics and Gynaecology at Monash University and a Research Fellow at Hudson Institute.

Professor Beverley Vollenhoven MBBS PhD FRANZCOG CREI is Deputy Head of the Department of Obstetrics and Gynaecology at Monash University, a clinician at Monash IVF, and Head of the Gynaecology unit at Monash Health.

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