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ARTICLE

Assessing receptivity in the endometrium: the need for a rapid, non-invasive test

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Abstract Successful implantation of an embryo into the uterus requires synchrony between the blastocyst and the endometrium. Endometrial preparedness, or receptivity, occurs only for a very short time during the mid-secretory phase of the menstrual cycle in fertile women. Failure to achieve receptivity results in infertility and is a rate-limiting step for IVF success. Frozen embryo transfer in non-stimulation cycles is already improving live birth rates. However, an important tool that is missing in the armoury of reproductive specialists is a means to rapidly assess endometrial receptivity, either during initial assessment or immediately prior to embryo transfer. The development of a wealth of omics technologies now opens the way for identifying potential receptivity markers, although validation of these is still a major issue. This review assesses the current state of the field and the requirements to proceed to a valid clinical test.

KEYWORDS: biomarker validation, endometrium, genomics, proteomics, receptivity, uterine fluid

Introduction

Implantation of the embryo into the womb is a critical event in establishing pregnancy. The initial steps in implantation must then proceed to formation of a sufficient placenta, with the capacity to support the fetus throughout pregnancy. From implantation and placental development until the maternal blood supply is tapped towards the end of the first trimester, there is a continuum of events in which each step depends on the adequacy of the previous one.

For successful implantation, there must be synchrony of development between the embryo and the endometrium: this was first observed in the 1960s during embryo transfer experiments in animals but also holds for women. The term applied to the endometrium when it is developmentally competent for implantation is that it is ''receptive''. The

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human endometrium becomes receptive to implantation in each normal menstrual cycle, following ovulation and driven by rising concentrations of both oestrogen and progesterone; this receptivity lasts for only about 4 days in the mid-secretory phase (Navot et al., 1991a,b). The current understanding of the mechanisms by which these steroids function to induce endometrial receptivity is reviewed in an accompanying article in this issue (Young, 2013). At the same time, the embryo, which has gone through considerable development during its passage through the Fallopian tube and entered the uterine cavity as an early blastocyst, is in developmental synchrony with the endometrium. During other cycle phases, the endometrium is either hostile or non-receptive to a blastocyst and implantation cannot occur. Failure of the endometrium to attain receptivity is one cause of infertility, and this is not currently assessed during infertility workup due to a lack of reliable markers for receptivity.

In addition to the normal cyclicity of the endometrium, it is clear that the hormonal treatments used to induce multiple ovulations in IVF clinics considerably disturb the endometrium. Premature secretory changes are seen in the post-ovulatory and early secretory phases of IVF cycles, followed by a considerable dys-synchronous glandular and stromal differentiation in the mid-luteal phase (Bourgain and Devroey, 2003; Kolibianakis and Devroey, 2002; Kolibianakis et al., 2002), resulting in both inadequate receptivity and/or changes in its timing. Importantly, in a recent comprehensive, retrospective study comparing morphological and immunohistochemical features on the day of oocyte retrieval in women in stimulation cycles, those women who became pregnant following embryo transfer in the same cycle had endometrium that was much less disturbed (significantly less alterations in histological endometrial maturation and the numbers and activation status of leukocyte populations) than that of the women who did not become pregnant (Evans et al., 2012b). The impact of ovarian stimulation on endometrial receptivity are reviewed in detail in an accompanying article in this issue (Fatemi and Popovic-Todorovic, 2013) Thus it is clear that the abnormal endometrial development seen in IVF cycles, limits the potential for implantation of even a developmentally competent embryo in that cycle. Indeed, pregnancy rates resulting from fresh embryo transfers consistently hover around 30% (www.cdc.gov/ART), and a wealth of data (Shapiro et al., 2011, 2013) now supports the contention that embryos have a better chance of implantation if they are frozen for subsequent transfer in a natural, unstimulated cycle. Furthermore, there is strong support for research leading to a test for endometrial receptivity that could be used in the clinic.

Current practices for embryo transfer

The surgical procedure to transfer the embryo into the endometrial cavity is vital to the successful outcome of the IVF treatment. First a speculum is placed in the vagina to visualize and clean the cervix. A transfer catheter is then loaded with the embryo(s) and inserted through the cervical canal into the uterine cavity. The embryos are then expelled approximately 1–2 cm from the uterine fundus.

Clinicians can control many variables to optimize the embryo transfer. These include the treatment of lower genital tract infections, correction of cervical stenosis during the fertility work up and the use of transabdominal ultrasound to guide the positioning of the transfer catheter (Mains and Van Voorhis, 2010).

The correct timing of the transfer is also critical for a successful outcome. Fresh or thawed embryos are typically transferred at the cleavage stage (day 2 or 3) or the blastocyst stage (day 5). The embryo transfer day is carefully selected such that the endometrium is in temporal synchrony with the developmental stage of the embryo: e.g. a day-2 or day-5 embryo will be transferred respectively on the second or the fifth day after the endometrium is first exposed to endogenous or exogenous progesterone.

It has been claimed in some studies that a premature rise of progesterone prior to the human chorionic gonadotrophin trigger is associated with a reduced pregnancy rate in stimulated cycles (Bosch et al., 2010; Huang et al., 2012; Ochsenkuhn et al., 2012), presumably because of the advancement of endometrial maturation relative to the developmental stage of the embryo. However, a meta-analysis by Venetis et al. (2007) concluded that a premature rise of progesterone was not correlated with pregnancy outcome (see also Fatemi and Popovic-Todorovic, 2013, in this issue).

Transvaginal ultrasound assessment of the endometrium can be used to describe the thickness, appearance and blood flow of the endometrium prior to embryo transfer. It is unclear how helpful these assessments are in determining whether the endometrium is optimally prepared. A systematic review and meta-analysis of 14 studies concluded that there may be a relationship between endometrial thickness and pregnancy, but that implantation potential is more complex than a single ultrasound measurement can determine (Momeni et al., 2011).

Clearly, better tests are required to assist the clinician with the decision when to defer a transfer and to freeze all embryos.

What clinicians need

Fertility specialists have for a long time been desperate to ensure embryos are transferred into a receptive endometrium. Access to a reliable endometrial receptivity assay would have at least three clinically useful applications.

The first group of women that would benefit are those with a history of repeated IVF failure. Women who have endured numerous failed IVF attempts with good-quality embryos are considered at an increased risk of having an endometrial receptivity disorder. In the absence of good tests to screen for such disorders, the decision to continue with further IVF treatment can be frustrating and difficult. Understanding which part of the dialogue between the embryo and the endometrium is dysfunctional would allow clinicians to better inform patients and to tailor their further treatment more specifically. Such diagnostic testing would occur in a standardized way during the window of implantation in the untreated cycle prior to the next IVF attempt. Repeat testing, especially in a controlled clinical trial setting, may also allow IVF specialists to assess which interventions are effective in optimizing endometrial

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receptivity. Women for whom a ''cure'' is not available can be advised appropriately and offered the option of surrogacy treatment.

The second application would broaden the use of the assay to all women undergoing IVF treatment. As discussed above, stimulated IVF cycles have a significant impact on the morphology (Bourgain and Devroey, 2003; Evans et al., 2012b; Kolibianakis et al., 2002) and the function of the endometrium while higher IVF success rates are achieved in women who are high responders to ovarian stimulation, if their frozen embryos are transferred in a subsequent natural cycle (Shapiro et al., 2011, 2013). These findings emphasize that altered hormonal environment in stimulated cycles is detrimental to endometrial receptivity. Thus an accurate test with a quick turn-around would allow IVF units to assess, for each patient individually, whether a fresh embryo transfer should go ahead or whether the patient should be advised to freeze her embryos. In addition to showing that the test is reliable, two conditions would thus need to be met: (i) the sampling (whether uterine lavage or endometrial biopsy) should not lower the chance of a subsequent implantation; and (ii) the assay should be able to be completed in the time frame between the sampling and the scheduled embryo transfer. Blastocyst transfers would have the advantage that samples may be taken closer to the opening of the window of implantation, increasing the probability that receptivity defects would be detected.

The third use of the test would be to broaden its application even further by incorporating it in the standard fertility work up. If the test is both reliable and affordable, there is no reason why screening for endometrial receptivity disorders should not occur on a larger scale before treatment starts. Treatment decisions will be influenced by the test results as treatment protocols tailored for endometrial receptivity disorders become available. Studies will have to confirm that data obtained in a natural cycle can be extrapolated in a meaningful way to the stimulated cycle.

Biomarker discovery and application

The English language has informally adopted the suffix omics to describe the advent of new technologies that enable analyses of broad systems within the study of biology. For example, the term ''transcriptomics'' applies to the analysis of the full set of the RNA transcribed by a cell or tissue. While omics technologies have been applied to biomarker discovery for some two decades, the recent speed of their development is now enabling rapid progress. Both genomic and proteomic approaches have recently yielded potentially clinically useful biomarkers of uterine receptivity: these markers are either clearly different between non-receptive and receptive endometrium or during the mid-secretory phase in women of proven fertility versus those who have had several failed IVF cycles with ''good'' embryos. While some of these biomarkers are already being applied either on a small commercial scale or in-house by certain clinics (Garrido-Gomez et al., 2013; Lessey, 2011), large-scale validation across many clinics has not been performed and the sensitivity and specificity of available tests is not known.

Endometrium-specific issues in discovery of biomarkers

Obtaining appropriate clinical material for studies is exceedingly difficult given the dynamic nature of the endometrium, in which the cellular and molecular composition changes on a daily basis. Accurate dating of the developmental stage of the endometrium by histological means is fraught with difficulties (Murray et al., 2004) and will remain subjective until molecular markers are widely available. A number of single and combined markers have been described since the late 1990s including molecules as diverse as integrins $\alpha v\beta 3$ (Lessey et al., 2000), nuclear pore proteins (Guffanti et al., 2008) and leukaemia inhibitory factor plus gp130 (Tawfeek et al., 2012). Furthermore, the recently described endometrial receptivity array, the ERA (Diaz-Gimeno et al., 2013), which is claimed to be more accurate than histological dating, suggests that this is now possible, although perhaps not in a suitable format for routine pathology use (Lessey, 2013). The availability of such a test indicates that it may now be possible to answer a number of important questions, provided the clinical material is available; a limitation not to be underestimated. These questions include: (i) what is the variation within the same women from cycle to cycle, in the timing of the onset of receptivity? (ii) what is the variation from woman to woman in this timing? (iii) does every normal fertile woman achieve endometrial receptivity in every cycle? (iv) which of the biomarkers used for defining normal receptivity are altered in women whose infertility is due to a failure to achieve receptivity? (v) are biomarkers different in women with different reasons for infertility (e.g. endometriosis and unexplained infertility)? and (vi) how dependent is endometrial receptivity on egg quality? On this last question, local effects of embryo products (particularly human chorionic gonadotrophin) on the endometrium are well documented (Licht et al., 2001; Paiva et al., 2011) and these appear to promote receptivity by increasing local production of important cytokines including leukaemia inhibitory factor and vascular endothelial growth factor. Furthermore, egg donation from young to older women has been shown to increase implantation rates to those seen in younger women (Navot et al., 1991a,b); it may be that these ''younger'' embryos send stronger local signals to the endometrium.

For the discovery phase for biomarkers of uterine receptivity, the ideal benchmark of normality would be marker concentrations in the mid-secretory phase of naturally cycling women of known fertility in their twenties or early thirties when fertility is optimum. A selected cohort of biomarker concentrations would most likely differ significantly from those in non-receptive endometrium of fertile women (proliferative or early-secretory phases) and/or from those in women in whom receptivity is known to be disturbed, such as the many women with endometriosis who are also infertile or women who have undergone multiple transfers of ''good'' embryos in an IVF programme. Subsequently, potential biomarkers would need to be tested in women presenting with infertility or during their treatment cycles in an IVF setting and be correlated with pregnancy outcomes in the same cycles. The stringent requirements and the substantial numbers of samples required for ''test'' and

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''validation'' sets will require considerable international collaboration. The selection of ''good-quality'' embryos for such studies is essential to distinguish between failure due to embryo quality and failure to develop receptive endometrium and is reviewed in this issue by Montag (2013).

Another important question is that of the best material on which to perform a molecular test. Options are tissue taken at curettage or by biopsy, uterine fluid taken by aspiration or lavage, blood plasma or serum and urine. While blood and urine are clearly more readily sampled, neither are likely to accurately reflect the status of the endometrium. Tissue has been widely used in this context and is relatively easy to obtain from parous women: however, many of the women needing testing will be nulliparous and access to the uterine cavity may be more difficult. Uterine fluid can be obtained less invasively than tissue but there are issues with consistency of recovery. Standardization is required between clinics and even between clinicians at single clinics, but this will clearly be difficult to achieve. Once clinical material is obtained, a range of analyses can be performed as outlined in **Figure 1**. While both genomics and proteomics have been the topic of a number of publications, lipidomics, glycomics, epigenomics and metabolomics are all very new approaches which offer considerable opportunities. For example, measurement of prostaglandins E and F2α (Vilella et al., 2013) has recently been applied to uterine fluid.

Genomic studies

Many global transcriptomic studies of human endometrium have been published in the past decade, providing considerable information on the likely pattern of expression in normal receptive endometrium. However, there are many differences between studies in the genes identified to change with receptivity, partly due to the different microarrays used for analysis and to differences in sample selection criteria and conditions. Thus consensus on a molecular signature of receptivity has not yet been achieved. The microarray studies fall into two major categories: those that focus entirely on (i) tissues from women of known fertility (normo-ovulatory women (Borthwick et al., 2003; Carson et al., 2002; Haouzi et al., 2009; Kao et al., 2002; Kuokkanen et al., 2010; Ponnampalam et al., 2004; Riesewijk et al., 2003; Talbi et al., 2006; Tseng et al., 2010) or fertile donors (Mirkin et al., 2005); (ii) those from fertile versus infertile women in the mid-secretory phase (Altmae et al., 2010; Koler et al., 2009); (iii) those that compare natural and stimulated cycles (Haouzi et al., 2012; Ruiz-Alonso et al., 2012); or (iv) tissue from women with repeated implantation failure or recurrent miscarriage (Ledee et al., 2011; Othman et al., 2012). The most useful of these studies in terms of discovery of biomarkers, are those comparing non-receptive (proliferative or early secretory phase) with receptive (mid-secretory phase) endometrium. The genes differentially expressed are both up- and down- regulated in the mid-secretory phase and many relate to known remodelling processes at this time: cell adhesion, metabolism, response to external stimuli, signalling, immune response and cell communication. As could be anticipated, transcription of genes related to cellular proliferation and development is decreased. The details of these findings have been recently reviewed in detail and tabulated (Haouzi et al., 2012; Ruiz-Alonso et al., 2012) and will not be repeated here.

One important limitation leading to the diversity of results in the studies detailed above is that endometrial tissue biopsies have been used for analysis. Endometrium contains many cell types including epithelial and stromal cells, cells of the vasculature and leukocytes and there is considerable heterogeneity of cellular composition across the cycle, particularly in terms of the ratio of glands to stroma and the number of leukocytes. Indeed, microarray analysis of individual cellular compartments obtained by laser capture showed that glands and stroma have distinct mRNA signatures, each dependent on the day of the cycle (Evans et al., 2012a). Use of laser-captured material in the initial discovery phase may be of use to overcome sensitivity issues. For example, SGK1, a kinase important for epithelial ion transport and cell survival, was identified as a potential marker of receptivity only in luminal epithelium (Salker et al., 2011), a minor component of the endometrium that is often lost during sampling and processing.

An important recent development from all these studies is the endometrial receptivity array (Diaz-Gimeno et al., 2013; Garrido-Gomez et al., 2013), in which 238 genes that are differentially regulated with the endometrial cycle, are customized on a single array. Of these, 134 genes represent a specific transcriptomic signature of the receptive phase. This test is of high specificity (0.8857) and sensitivity (0.9976) for endometrial dating: its ability to correlate molecular data with clinical outcomes is still under investigation.

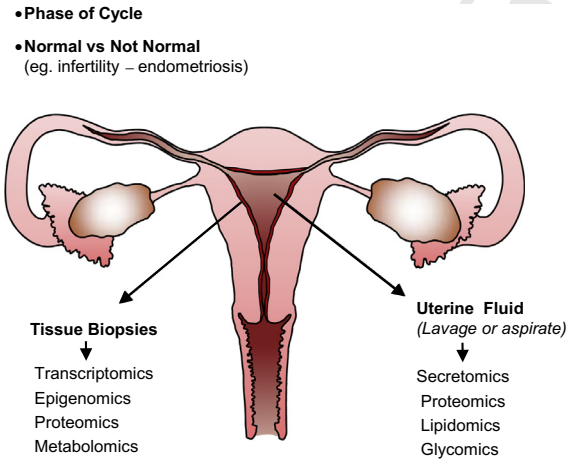

Figure 1 Potential approaches for discovery of markers for receptivity. Sampling of either endometrial tissue or of uterine fluid must take into account the phase of the cycle and known endometrial disorders. Differential analyses using a range of omics have the potential to provide a wealth of markers for further validation.

Proteomics

Proteomics, or the analysis of the proteins in any sample, provides more physiologically relevant information than

does genomics, since there are many regulatory steps between the transcriptome and functional proteins: indeed correlation between an mRNA and its protein derivative in the endometrium is often low (Burney et al., 2007; Chen ?tul?> et al., 2009; Haouzi et al., 2012). While this is not necessarily important in terms of provision of biomarkers, transcriptomic data can be misleading in terms of providing leads to function. Proteomic analyses to date, mostly utilizing two-dimensional differential in-gel electrophoresis (2D-DiGE) followed by matrix-assisted desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF MS) have identified a number of protein changes between the proliferative and secretory phases (Chen et al., 2009; DeSouza et al., 2005; Parmar et al., 2009) or between pre-receptive and receptive endometrium (Dominguez et al., 2009; Li et al., 2006). Some of these proteins have been validated by other means and immunohistochemistry has confirmed their presence and cellular location in the endometrium. The differentially produced proteins identified by these techniques and subsequently validated are listed in **Table 1** and have been detailed further in recent reviews (Haouzi et al., 2012; Salamonsen et al., 2012). A major limitation to date has been that the most abundant proteins in the samples are structural and these mask lower abundance regulatory proteins. In addition, gel separation is not ideal for proteins of low molecular weight (<20 kDa). Furthermore, with the development of highly sensitive mass spectrometers, it is now apparent that most protein spots cut from gels represent a number of proteins. Pre-fractionation of samples and application of newer gel-free techniques to such samples is likely to provide many more useful markers in coming years.

Uterine fluid is a protein-rich histotroph that contains, among other components, secretions from the endometrial glands and cleavage products of both the secreted proteins and the glycocalyx (the glycoprotein mucin-rich layer coating the endometrial apical cell surface). Importantly, uterine fluid provides the microenvironment for the final stages of blastocyst development and implantation. Glandular secretions are essential for implantation in both sheep (Gray et al., 2001) and mice (Dunlap et al., 2011; Jeong et al., 2010), as demonstrated in animals in which uterine gland development was inhibited during early post-natal life. Given that many of the endometrial molecules known to be important for implantation in women are produced and secreted from the glands (Salamonsen et al., 2009), it is clear that proteomic analysis of uterine fluid should provide biomarkers for endometrial receptivity. However, uterine

Table 1 Validated proteins of relevance to endometrial receptivity, discovered by proteomic approaches in endometrial tissues from cycling women of proven fertility.

Annotation	*Protein*	*Comparison*	*Time of biopsy*	*Direction of change versus MS*[a]	*Validation method*	*Cellular location*	*Reference*
CLIC1_HUMAN	Chloride intracellular channel protein 1	MP versus MS	Days 9–11 versus days 19–23	↓	IHC	Epithelium	Chen et al. (2009)
GDIR1_HUMAN	Rho GDP-dissociation inhibitor 1	MP versus MS	Days 9–11 versus days 19–23	↓		Epithelium	Chen et al. (2009)
PGRC1_HUMAN	Membrane-associated progesterone receptor component 1	MP versus MS	Days 9–11 versus days 19–23	↓	IHC	Stroma	Chen et al. (2009)
EZRI_HUMAN	Ezrin	MP versus MS	Days 9–11 versus days 19–23	↓	IHC	Epithelium	Chen et al. (2009), Heng et al. (2011)
STMN1_HUMAN	Stathmin 1	Pre-receptive versus receptive	Day LH+2 versus day LH+7	↓	IHC, WB	Mostly stroma	Dominguez et al. (2009)
ANXA2_HUMAN	Annexin A2	Pre-receptive versus receptive	Day LH+2 versus day LH+7	↑	IHC, WB	Epithelium and stroma	Dominguez et al. (2009)
ANXA4_HUMAN	AnnexinIV	Pre-receptive versus receptive	Day LH+2 versus day LH+7	↓	IHC	Epithelium	Li et al. (2006), Chen et al. (2009))
CAL_HUMAN	Calreticulum	MP versus MS		↑	IHC	Epithelium and stroma	Parmar et al. (2009)
AIAT_HUMAN	α1-Antitrypsin	MP versus MS		↑	IHC	Epithelium and stroma	Parmar et al. (2009)

IHC = immunohistochemistry; MP = mid-proliferative; MS = mid-secretory; WB = Western blot.
[a]In each case, fold change > 1.4; ↑ = increase; ↓ = decrease.

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fluid also contains abundant serum proteins (Hannan et al., 2009) which may be selectively transudated from blood and which provide ~90% of the total protein in uterine fluid. These can mask the less abundant proteins in uterine fluid and are often removed prior to proteomic analysis.

Proteomic approaches have started to define the overall protein composition of secretory phase uterine fluid (Casado-Vela et al., 2009) and have examined differences both between proliferative or early secretory phases and the mid-secretory phase (Hannan et al., 2010; Scotchie et al., 2009) and between the secretory phase in fertile and infertile women (Hannan et al., 2010). Casada-Vela identified 803 separate proteins in uterine fluid, after applying three different strategies to uterine aspirates; these proteins included many proteins which are found in blood. Whether they are expressed and secreted from within the endometrial tissue or transudated from blood needs to be established. Several proteins have been identified as associated with endometrial cyclicity or embryo implantation; mucin 1, vitamin D binding protein, apolipoprotein A1, alpha-1 antitrypsin, matrix metalloproteinase 9 (MMP9) and TIMP1; many of these are also found in blood. It is likely that MMP9 at least is derived from the MMP9-positive leukocytes within the fluid. Such cells were removed prior to other analyses of lavage samples (Hannan et al., 2010; Scotchie et al., 2009). Alpha-1 antitrypsin was common to all three studies, being decreased in the mid-secretory phase. Antithrombin III and alpha-2-macroglobulin were the only proteins among these cohorts whose expression was further examined in mid-secretory tissue: both were epithelial products indicating these secretory cells as being their likely source of the fluid proteins. The intensity of epithelial staining was also correlated with their changes in uterine fluid across the cycle and in infertile women, validating the proteomic data. It should be stressed that such laboratory analyses are performed on a relatively small number of samples: in due course, biomarker candidates will need to be assessed in larger clinical cohorts.

Multiplex analyses have proven useful to address the issue of lower-molecular-weight biological mediators that are not readily detected in gel-based broad proteomic analyses. Simultaneous analysis of up to 42 individual analytes (particularly chemokines and cytokines) in uterine fluid has identified some known and others previously unknown in this setting, that differ depending on the fertility status of the woman (Hannan et al., 2010, 2011) or that are predictive of pregnancy outcome (Boomsma et al., 2009a,b). One limitation of this technique is the cross-reactivity of the analytes on the array, although this can eventually be overcome by use of new well-screened monoclonal antibodies. For example, leukaemia inhibitory factor, an obvious choice for analysis, could not be assayed together with the other components of either multiplex system (Boomsma et al., 2009a,b; Hannan et al., 2010). The advantage of multiplexing is that only very small volumes of fluid are required for simultaneous analysis of the many analytes in a relatively short timeframe, making it an excellent platform for biomarker applications.

A major advantage of uterine fluid over endometrial tissue for assessment of receptivity is that uterine fluid is readily available by less destructive means than is biopsy. Fluid may be harvested by aspiration (provides only 1–5 μl total volume) (Boomsma et al., 2009a,b; Casado-Vela et al., 2009) or by lavage with saline solution (Hannan et al., 2011, 2010; Scotchie et al., 2009). Each method presents advantages and disadvantages (Hannan et al., 2012). Both have variable recovery and contain some leukocytes and endometrial cells. While these can and must be removed immediately by gentle centrifugation of the sample, they may well have released proteins prior to or during sampling. Importantly, analysis of the same set of eight markers in matched aspirates and lavages clearly demonstrated that the rank concentrations of the markers differed between the two fluids, emphasizing that either one or the other method must be selected for analysis and that they cannot be interchanged. At least some of the difference will result from the washing of the uterine surface during the lavage, and the likely dislodging of soluble molecules either loosely bound to or entrapped in the glycocalyx; indeed, lavage provides around 10-fold more protein for analysis than does aspiration. In this study group's hands, the aspirates often contained blood, which can interfere with subsequent assays. However, aspiration may be the better technique if sampling is to be performed in the same cycle as embryo transfer as it has been proven not to interfere with implantation rates (van der Gaast et al., 2003).

It would clearly be preferable to analyse either serum/plasma or urine for a routine and readily applicable test for receptivity. The likelihood of this being successful is not high given the local production of most potential receptivity markers. Indeed, given that very few if any mediators are specific to the endometrium and that most act in a paracrine or autocrine manner, it is likely that changes in the receptive endometrium would be undetectable or would be subject to interference by many physiological events or pathologies, including even mild inflammation. A recent promising development in this regard relates to reports that circulating micro-RNA (miRNA), derived from the endometrium, are detectable in peripheral blood. Concentrations of selected miRNA could provide non-invasive diagnostic information regarding endometrial function and are an active area of research (Hull and Nisenblat, 2013, in this issue).

Validation

For a handful of identified potential receptivity markers, the authors have provided evidence of their endometrial expression, in the form of immunohistochemistry, primary cell culture and, in some cases, immunoassay of small cohorts of individuals. While this provides some supporting evidence to further pursue these markers, it remains for any of these potential markers to be assessed across extensive cohorts of well-defined women.

Proteomic studies to date of either tissue (Brosens et al., 2010; Dominguez et al., 2009) or flushing/lavage (Casado-Vela et al., 2009; Hannan et al., 2009; Parmar et al., 2008) have provided extensive lists of proteins generated and secreted by the endometrium. Some authors have analysed individual samples while others have elected to utilize sample pooling. Commonly however, they have all used only small sample sets of between 3 and 15 per group for analysis. Thus, the results must always be regarded with

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some caution since, with the small cohorts of samples used in the analyses, the risk of a type I error is significant. Indeed this is highlighted by Dominguez et al. (2009) when comparing tissue from ovum donors on days LH+2 and LH+7. In duplicate experiments, using separate pools each comprising three individuals, only two proteins were common to the two data sets generated. This highlights the need for independent validation of proposed markers whether they have originated as a result of proteomic studies or through other methodologies.

Paramount to validating a biomarker for clinical use is the need to clearly define just how it will be used. Selected cohorts and technologies must be appropriate and applicable to clinical needs. As discussed above, a receptivity test may find wide applicability as a screening test for all infertile women during their work up to establish their ability to generate a receptive endometrium. This situation would not require a rapid assay; however, the alternative scenario of testing receptivity as a decision point on whether to use fresh or frozen embryo transfer would require a quicker test result. Although many current analyses (e.g. ELISA), require hours as opposed to minutes, advances in point of care assay technologies may afford the luxury of testing within minutes of a planned embryo transfer. However, this would require a clear margin between receptive and non-receptive data. The positive and negative predictive value of the receptivity test will also change with the prevalence of receptivity disorders in the population being tested. As such, a diagnostic test that is reliable for patients with repeat implantation failure may not be an appropriate test as part of the initial work up of all infertile women.

Any validation cohort must also reflect the final test application. If the test is to be used for IVF cycle testing. It is essential that this group is included in such validation, since the morphology of an IVF cycle endometrium is greatly changed from that of a natural cycle. Validation must also consider potential interferences. It is essential that validation cohorts do not exclude conditions which are commonly present in the potential patient group (e.g. fibroids, mild endometriosis), but rather includes them and identifies any potential patient groups which must be excluded. The control cohort is also problematic, given that to date there is no evidence that fertile women achieve receptivity in every cycle. Thus the use of women of proven fertility is questionable, particularly since it may be a number of years since that woman last conceived. Given such issues, full characterization and validation of a receptivity test could be problematic. However, the nature of the target does afford prospective testing (successful versus unsuccessful embryo transfer), since samples and outcomes are available within a relatively short timeframe.

Final validation studies must incorporate sufficient samples to provide robust evidence of utility, to enable determination of sensitivity and specificity and to provide clear evidence of assay performance (e.g. precision, accuracy and importantly external validity). Multi-site collection and performance are paramount to proving robustness of any test. Indeed, as highlighted above, sampling techniques can be variable. Biopsies provide mixed cell types in different proportions, while flushings/lavage are subject to blood and antiseptic contamination and volume issues. The latter may be overcome by use of analyte ratios to normalize for sample volume variation, given the current absence of any defined control marker whose expression is unchanged across the cycle and between women.

It is not likely that a single biomarker will suffice to identify receptive endometrium due to the complexity both of infertility and of individuality. It is most likely that a multiple marker panel approach will emerge and afford personalized treatment of individual woman in IVF clinics. In 2009, the US Food and Drug Administration approved the first in-vitro diagnostic multivariate index assay (IVDMIA); others have followed for application in disorders including ovarian and breast cancer and pre-eclampsia. An IVDMIA allows the utilization of multiple biomarkers whose combined diagnostic efficiency is greater than any of their individual performances. Multiple marker panels may be applied to a range of platforms including the antibody-based multiplex or a mass spectrometer. While the use of multiple markers has been practised by physicians for many years, the complex computational modelling of the IVDMIA sets it apart. The technical and regulatory aspects of the application of multiple marker diagnostics are reviewed elsewhere (Ellington et al., 2009; Rodriguez et al., 2010; Zhang, 2012). While these tests provide improvement beyond the performance of any single analyte on offer, there are important additional validation requirements to consider. These include potential cross interference between analytes in platforms such as multiplex ELISA and a requirement for multiple site participation to eliminate potential artefactual patterns due to specific sampling procedures. However, the primary difference is the greater sample numbers required to provide both a ''modelling'' or ''learning'' sample set to provide a diagnostic signature and a ''validation'' set for testing performance of the signature.

Future directions

It is increasingly clear that single biomarkers are not likely to exist or to be widely useful in most clinical contexts, since the complexity of any disease or specific physiological state precludes its identification by a single biomarker. Indeed, scepticism over single markers has been expressed by many regulatory agencies and clinicians. The most useful overall strategy and one that certainly applies to endometrial receptivity is that a cohort of markers will be required in spite of the difficulties this represents in terms of validation.

Although there are thought to be some 23,000 genes in the human genome, this pales in comparison with the size and diversity of the proteome, which is predicted to be more than 1 million proteins (Walsh et al., 2005). Furthermore, each primary gene product is likely to exist as the combinatorial sum of a number of post-translationally modified forms (Seo et al., 2008) of which >200 have been identified. While the most studied post-translationally modified form is reversible post-translational phosphorylation, protein lysine acetylation, ubiquitination and SUMOylation are also common (Thelen and Miernyk, 2012). With particular respect to endometrial proteins, glycosylation, which potentially presents a myriad of different forms of a protein, is very common as shown by the ''trains'' of spots seen on two-dimensional gels. Indeed, in a study on endometri-

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osis, one protein examined differed in only one of a number of glycoforms (Stephens et al., 2010). The rapidly developing field of glycomics is therefore highly applicable to biomarker discovery programmes. Proteins can also be modified by enzymatic cleavage resulting in activation, altered specificity, potency or function. Proprotein convertases and MMP, both found in the endometrium (Freyer et al., 2007) and in the uterine cavity, are highly effective in such post-translationally modified forms.

The analysis of lipids, or lipidomics, is another approach that has been largely ignored until recently (Simon, 2012). While the lipids studied to date in uterine fluid are the prostaglandins E and F2α, which have known roles in implantation (Sordelli et al., 2012; Wang and Dey, 2005), a more global approach to lipid analysis of endometrium, could provide additional biomarkers.

Regardless of the biomarker cohorts selected to identify a receptive endometrium, all will require broad validation before their clinical utility can be proven. Such validation should include: (i) large cohorts of patients of normal fertility, so as to understand and quantify a ''normal range'' of variation, whether there is race- or age-related variation; (ii) further testing of markers that apparently distinguish receptive or non-receptive endometrium, in additional clinical cohorts to ensure their reproducibility between different clinical and laboratory settings; (iii) prospective studies to determine whether the biomarkers can be used to inform clinicians in IVF or other settings; for example, whether they are beneficial in decision making as to whether to proceed to embryo transfer in a retrieval cycle or rather to freeze for later transfer; and (iv) assessment in women with different underlying causes (e.g. endometriosis or not) as biomarker efficacy may differ in such cohorts.

In the longer term, a point-of-care diagnostic with rapid turnaround of results will be needed if embryos are to be transferred in the cycle of testing. Ideally this will be a day LH+2 (or earlier) test that predicts that the endometrium will become receptive in this cycle. Such development will need to follow the identification and validation of markers.

Conclusion

While a number of potential biomarkers of endometrial receptivity are now available, international collaboration will be required to sufficiently validate the optimum cohort of these and to bring a robust test to the marketplace and the clinic. Sampling techniques will need to be simplified and widely applicable. Proof will be required of the impact of receptivity testing on clinical practice, on how infertility is assessed and on outcomes of IVF cycles in which decision making has been informed by such a test. For major impact, the test will need to be provided as an in-clinic platform that provides the results quickly. However, given the rapidly increasing numbers of couples presenting with infertility, there is a strong imperative to deliver.

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References

Altmae, S., Martinez-Conejero, J.A., Salumets, A., Simon, C., Horcajadas, J.A., Stavreus-Evers, A., 2010. Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility. Mol. Hum. Reprod. 16, 178–187.

Boomsma, C.M., Kavelaars, A., Eijkemans, M.J., Amarouchi, K., Teklenburg, G., Gutknecht, D., Fauser, B.J., Heijnen, C.J., Macklon, N.S., 2009a. Cytokine profiling in endometrial secretions: a non-invasive window on endometrial receptivity. Reprod. Biomed. Online 18, 85–94.

Boomsma, C.M., Kavelaars, A., Eijkemans, M.J., Lentjes, E.G., Fauser, B.C., Heijnen, C.J., Macklon, N.S., 2009b. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum. Reprod. 24, 1427–1435.

Borthwick, J.M., Charnock-Jones, D.S., Tom, B.D., Hull, M.L., Teirney, R., Phillips, S.C., Smith, S.K., 2003. Determination of the transcript profile of human endometrium. Mol. Hum. Reprod. 9, 19–33.

Bosch, E., Labarta, E., Crespo, J., Simon, C., Remohi, J., Jenkins, J., Pellicer, A., 2010. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum. Reprod. 25, 2092–2100.

Bourgain, C., Devroey, P., 2003. The endometrium in stimulated cycles for IVF. Hum. Reprod. Update 9, 515–522.

Brosens, J.J., Hodgetts, A., Feroze-Zaidi, F., Sherwin, J.R., Fusi, L., Salker, M.S., Higham, J., Rose, G.L., Kajihara, T., Young, S.L., Lessey, B.A., Henriet, P., Langford, P.R., Fazleabas, A.T., 2010. Proteomic analysis of endometrium from fertile and infertile patients suggests a role for apolipoprotein A–I in embryo implantation failure and endometriosis. Mol. Hum. Reprod. 16, 273–285.

Burney, R.O., Talbi, S., Hamilton, A.E., Vo, K.C., Nyegaard, M., Nezhat, C.R., Lessey, B.A., Giudice, L.C., 2007. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology 148, 3814–3826.

Carson, D.D., Lagow, E., Thathiah, A., Al-Shami, R., Farach-Carson, M.C., Vernon, M., Yuan, L., Fritz, M.A., Lessey, B., 2002. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. Mol. Hum. Reprod. 8, 871–879.

Casado-Vela, J., Rodriguez-Suarez, E., Iloro, I., Ametzazurra, A., Alkorta, N., Garcia-Velasco, J.A., Matorras, R., Prieto, B., Gonzalez, S., Nagore, D., Simon, L., Elortza, F., 2009. Comprehensive proteomic analysis of human endometrial fluid aspirate. J. Proteome Res. 8, 4622–4632.

Chen, J.I., Hannan, N.J., Mak, Y., Nicholls, P.K., Zhang, J., Rainczuk, A., Stanton, P.G., Robertson, D.M., Salamonsen, L.A., Stephens, A.N., 2009. Proteomic characterization of midproliferative and midsecretory human endometrium. J. Proteome Res. 8, 2032–2044.

DeSouza, L., Diehl, G., Yang, E.C., Guo, J., Rodrigues, M.J., Romaschin, A.D., Colgan, T.J., Siu, K.W., 2005. Proteomic analysis of the proliferative and secretory phases of the human endometrium: protein identification and differential protein expression. Proteomics 5, 270–281.

Diaz-Gimeno, P., Ruiz-Alonso, M., Blesa, D., Bosch, N., Martinez-Conejero, J.A., Alama, P., Garrido, N., Pellicer, A., Simon,

C., 2013. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. Fertil. Steril. 99, 508–517.

Dominguez, F., Garrido-Gomez, T., Lopez, J.A., Camafeita, E., Quinonero, A., Pellicer, A., Simon, C., 2009. Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated. Hum. Reprod. 24, 2607–2617.

Dunlap, K.A., Filant, J., Hayashi, K., Rucker 3rd, E.B., Song, G., Deng, J.M., Behringer, R.R., DeMayo, F.J., Lydon, J., Jeong, J.W., Spencer, T.E., 2011. Postnatal deletion of Wnt7a inhibits uterine gland morphogenesis and compromises adult fertility in mice. Biol. Reprod. 85, 386–396.

Ellington, A.A., Kullo, I.J., Bailey, K.R., Klee, G.G., 2009. Measurement and quality control issues in multiplex protein assays: a case study. Clin. Chem. 55, 1092–1099.

Evans, G.E., Martinez-Conejero, J.A., Phillipson, G.T., Simon, C., McNoe, L.A., Sykes, P.H., Horcajadas, J.A., Lam, E.Y., Print, C.G., Sin, I.L., Evans, J.J., 2012a. Gene and protein expression signature of endometrial glandular and stromal compartments during the window of implantation. Fertil. Steril. 97, 1365–1373.

Evans, J., Hannan, N.J., Hincks, C., Rombauts, L.J., Salamonsen, L.A., 2012b. Defective soil for a fertile seed? Altered endometrial development is detrimental to pregnancy success. PLoS ONE 7, e53098.

Fatemi, H.M., Popovic-Todorovic, B., 2013. Implantation in ART: a look at endometrial receptivity. Reprod. Biomed. Online.

Freyer, C., Kilpatrick, L.M., Salamonsen, L.A., Nie, G., 2007. Pro-protein convertases (PCs) other than PC6 are not tightly regulated for implantation in the human endometrium. Reproduction 133, 1189–1197.

Garrido-Gomez, T., Ruiz-Alonso, M., Blesa, D., Diaz-Gimeno, P., Vilella, F., Simon, C., 2013. Profiling the gene signature of endometrial receptivity: clinical results. Fertil. Steril. 99, 1078–1085.

Gray, C.A., Taylor, K.M., Ramsey, W.S., Hill, J.R., Bazer, F.W., Bartol, F.F., Spencer, T.E., 2001. Endometrial glands are required for preimplantation conceptus elongation and survival. Biol. Reprod. 64, 1608–1613.

Guffanti, E., Kittur, N., Brodt, Z.N., Polotsky, A.J., Kuokkanen, S.M., Heller, D.S., Young, S.L., Santoro, N., Meier, U.T., 2008. Nuclear pore complex proteins mark the implantation window in human endometrium. J. Cell Sci. 121, 2037–2045.

Hannan, N.J., Stoikos, C.J., Stephens, A.N., Salamonsen, L.A., 2009. Depletion of high-abundance serum proteins from human uterine lavages enhances detection of lower-abundance proteins. J. Proteome Res. 8, 1099–1103.

Hannan, N.J., Stephens, A.N., Rainczuk, A., Hincks, C., Rombauts, L.J., Salamonsen, L.A., 2010. 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women. J. Proteome Res. 9, 6256–6264.

Hannan, N.J., Paiva, P., Meehan, K.L., Rombauts, L.J., Gardner, D.K., Salamonsen, L.A., 2011. Analysis of fertility-related soluble mediators in human uterine fluid identifies VEGF as a key regulator of embryo implantation. Endocrinology 152, 4948–4956.

Hannan, N.J., Nie, G., Rainzcuk, A., Rombauts, L.J., Salamonsen, L.A., 2012. Uterine lavage or aspirate: which view of the intrauterine environment? Reprod. Sci. 19, 1125–1132.

Haouzi, D., Assou, S., Mahmoud, K., Tondeur, S., Reme, T., Hedon, B., De Vos, J., Hamamah, S., 2009. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. Hum. Reprod. 24, 1436–1445.

Haouzi, D., Dechaud, H., Assou, S., De Vos, J., Hamamah, S., 2012. Insights into human endometrial receptivity from transcriptomic and proteomic data. Reprod. Biomed. Online 24, 23–34.

Heng, S., Cervero, A., Simon, C., Stephens, A.N., Li, Y., Zhang, J., Paule, S., Rainczuk, A., Singh, H., Quinonero, A., Tapia, A., Velasquez, L., Salamonsen, L., Rombauts, L.J., Nie, G., 2011. Proprotein convertase 5/6 is critical for embryo implantation in women: regulating receptivity by cleaving EBP50, modulating ezrin binding and membrane–cytoskeletal interactions. Endocrinology 152, 5041–5052.

Huang, R., Fang, C., Xu, S., Yi, Y., Liang, X., 2012. Premature progesterone rise negatively correlated with live birth rate in IVF cycles with GnRH agonist: an analysis of 2,566 cycles. Fertil. Steril. 98, 664–670.

Hull, M.L., Nisenblat, V., 2013. Tissue and circulating MicroRNAs influence reproductive function in endometrial disease. Reprod. Biomed. Online.

Jeong, J.W., Kwak, I., Lee, K.Y., Kim, T.H., Large, M.J., Stewart, C.L., Kaestner, K.H., Lydon, J.P., DeMayo, F.J., 2010. Foxa2 is essential for mouse endometrial gland development and fertility. Biol. Reprod. 83, 396–403.

Kao, L.C., Tulac, S., Lobo, S., Imani, B., Yang, J.P., Germeyer, A., Osteen, K., Taylor, R.N., Lessey, B.A., Giudice, L.C., 2002. Global gene profiling in human endometrium during the window of implantation. Endocrinology 143, 2119–2138.

Koler, M., Achache, H., Tsafrir, A., Smith, Y., Revel, A., Reich, R., 2009. Disrupted gene pattern in patients with repeated in vitro fertilization (IVF) failure. Hum. Reprod. 24, 2541–2548.

Kolibianakis, E.M., Devroey, P., 2002. The luteal phase after ovarian stimulation. Reprod. Biomed. Online 5, 26–35.

Kolibianakis, E., Bourgain, C., Albano, C., Osmanagaoglu, K., Smitz, J., Van Steirteghem, A., Devroey, P., 2002. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. Fertil. Steril. 78, 1025–1029.

Kuokkanen, S., Chen, B., Ojalvo, L., Benard, L., Santoro, N., Pollard, J.W., 2010. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. Biol. Reprod. 82, 791–801.

Ledee, N., Munaut, C., Aubert, J., Serazin, V., Rahmati, M., Chaouat, G., Sandra, O., Foidart, J.M., 2011. Specific and extensive endometrial deregulation is present before conception in IVF/ICSI repeated implantation failures (IF) or recurrent miscarriages. J. Pathol. 225, 554–564.

Lessey, B.A., 2011. Assessment of endometrial receptivity. Fertil. Steril. 96, 522–529.

Lessey, B.A., 2013. The pathologists are free to go, or are they? Fertil. Steril. 99, 350–351.

Lessey, B.A., Castelbaum, A.J., Wolf, L., Greene, W., Paulson, M., Meyer, W.R., Fritz, M.A., 2000. Use of integrins to date the endometrium. Fertil. Steril. 73, 779–787.

Li, J., Tan, Z., Li, M.T., Liu, Y.L., Liu, Q., Gu, X.F., Zhou, J.Z., Zhuang, G.L., 2006. Study of altered expression of annexin IV and human endometrial receptivity. Zhonghua fu chan ke za zhi 41, 803–805.

Licht, P., Russu, V., Wildt, L., 2001. On the role of human chorionic gonadotropin (hCG) in the embryo–endometrial microenvironment: implications for differentiation and implantation. Semin. Reprod. Med. 19, 37–47.

Mains, L., Van Voorhis, B.J., 2010. Optimizing the technique of embryo transfer. Fertil. Steril. 94, 785–790.

Mirkin, S., Arslan, M., Churikov, D., Corica, A., Diaz, J.I., Williams, S., Bocca, S., Oehninger, S., 2005. In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum. Reprod. 20, 2104–2117.

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Momeni, M., Rahbar, M.H., Kovanci, E., 2011. A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles. J. Hum. Reprod. Sci. 4, 130–137.

Montag, M.M., 2013. New approaches to embryo selection. Reprod. Biomed. Online.

Murray, M.J., Meyer, W.R., Zaino, R.J., Lessey, B.A., Novotny, D.B., Ireland, K., Zeng, D., Fritz, M.A., 2004. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertil. Steril. 81, 1333–1343.

Navot, D., Bergh, P.A., Williams, M., Garrisi, G.J., Guzman, I., Sandler, B., Fox, J., Schreiner-Engel, P., Hofmann, G.E., Grunfeld, L., 1991a. An insight into early reproductive processes through the in vivo model of ovum donation. J. Clin. Endocrinol. Metab. 72, 408–414.

Navot, D., Bergh, P.A., Williams, M.A., Garrisi, G.J., Guzman, I., Sandler, B., Grunfeld, L., 1991b. Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 337, 1375–1377.

Ochsenkuhn, R., Arzberger, A., von Schonfeldt, V., Gallwas, J., Rogenhofer, N., Crispin, A., Thaler, C.J., Noss, U., 2012. Subtle progesterone rise on the day of human chorionic gonadotropin administration is associated with lower live birth rates in women undergoing assisted reproductive technology: a retrospective study with 2555 fresh embryo transfers. Fertil. Steril. 98, 347–354.

Othman, R., Omar, M.H., Shan, L.P., Shafiee, M.N., Jamal, R., Mokhtar, N.M., 2012. Microarray profiling of secretory-phase endometrium from patients with recurrent miscarriage. Reprod. Biol. 12, 183–199.

Paiva, P., Hannan, N.J., Hincks, C., Meehan, K.L., Pruysers, E., Dimitriadis, E., Salamonsen, L.A., 2011. Human chorionic gonadotrophin regulates FGF2 and other cytokines produced by human endometrial epithelial cells, providing a mechanism for enhancing endometrial receptivity. Hum. Reprod. 26, 1153–1162.

Parmar, T., Sachdeva, G., Savardekar, L., Katkam, R.R., Nimbkar-Joshi, S., Gadkar-Sable, S., Salvi, V., Manjramkar, D.D., Meherji, P., Puri, C.P., 2008. Protein repertoire of human uterine fluid during the mid-secretory phase of the menstrual cycle. Hum. Reprod. 23, 379–386.

Parmar, T., Gadkar-Sable, S., Savardekar, L., Katkam, R., Dharma, S., Meherji, P., Puri, C.P., Sachdeva, G., 2009. Protein profiling of human endometrial tissues in the midsecretory and proliferative phases of the menstrual cycle. Fertil. Steril. 92, 1091–1103.

Ponnampalam, A.P., Weston, G.C., Trajstman, A.C., Susil, B., Rogers, P.A., 2004. Molecular classification of human endometrial cycle stages by transcriptional profiling. Mol. Hum. Reprod. 10, 879–893.

Riesewijk, A., Martin, J., van Os, R., Horcajadas, J.A., Polman, J., Pellicer, A., Mosselman, S., Simon, C., 2003. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol. Hum. Reprod. 9, 253–264.

Rodriguez, H., Tezak, Z., Mesri, M., Carr, S.A., Liebler, D.C., Fisher, S.J., Tempst, P., Hiltke, T., Kessler, L.G., Kinsinger, C.R., Philip, R., Ransohoff, D.F., Skates, S.J., Regnier, F.E., Anderson, N.L., Mansfield, E., Workshop Participants, 2010. Analytical validation of protein-based multiplex assays: a workshop report by the NCI–FDA interagency oncology task force on molecular diagnostics. Clin. Chem. 56, 237–243.

Ruiz-Alonso, M., Blesa, D., Simon, C., 2012. The genomics of the human endometrium. Biochim. Biophys. Acta 1822, 1931–1942.

Salamonsen, L.A., Nie, G., Hannan, N.J., Dimitriadis, E., 2009. Society for Reproductive Biology Founders' Lecture 2009. Preparing fertile soil: the importance of endometrial receptivity. Reprod. Fertil. Dev. 21, 923–934.

Salamonsen, L.A., Edgell, T., Rombauts, L.J., Stephens, A.N., Robertson, D.M., Rainczuk, A., Nie, G., Hannan, N.J., 2012. Proteomics of the human endometrium and uterine fluid: a pathway to biomarker discovery. Fertil. Steril. 99, 1086–1092.

Salker, M.S., Christian, M., Steel, J.H., Nautiyal, J., Lavery, S., Trew, G., Webster, Z., Al-Sabbagh, M., Puchchakayala, G., Foller, M., Landles, C., Sharkey, A.M., Quenby, S., Aplin, J.D., Regan, L., Lang, F., Brosens, J.J., 2011. Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. Nat. Med. 17, 1509–1513.

Scotchie, J.G., Fritz, M.A., Mocanu, M., Lessey, B.A., Young, S.L., 2009. Proteomic analysis of the luteal endometrial secretome. Reprod. Sci. 16, 883–893.

Seo, J., Jeong, J., Kim, Y.M., Hwang, N., Paek, E., Lee, K.J., 2008. Strategy for comprehensive identification of post-translational modifications in cellular proteins, including low abundant modifications: application to glyceraldehyde-3-phosphate dehydrogenase. J. Proteome Res. 7, 587–602.

Shapiro, B.S., Daneshmand, S.T., Garner, F.C., Aguirre, M., Hudson, C., Thomas, S., 2011. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. Fertil. Steril. 96, 516–518.

Shapiro, B.S., Daneshmand, S.T., Restrepo, H., Garner, F.C., Aguirre, M., Hudson, C., 2013. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. Fertil. Steril. 99, 389–392.

Simon, C., 2012. Profiling the gene signature of endometrial receptivity. In: The 1st Biomarker Meeting in Reproductive Medicine: Emergence of a New Field, Valencia, Spain, pp. S-6.

Sordelli, M.S., Beltrame, J.S., Cella, M., Gervasi, M.G., Perez Martinez, S., Burdet, J., Zotta, E., Franchi, A.M., Ribeiro, M.L., 2012. Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus. PLoS ONE 7, e46059.

Stephens, A.N., Hannan, N.J., Rainczuk, A., Meehan, K.L., Chen, J., Nicholls, P.K., Rombauts, L.J., Stanton, P.G., Robertson, D.M., Salamonsen, L.A., 2010. Post-translational modifications and protein-specific isoforms in endometriosis revealed by 2D DIGE. J. Proteome Res. 9, 2438–2449.

Talbi, S., Hamilton, A.E., Vo, K.C., Tulac, S., Overgaard, M.T., Dosiou, C., Le Shay, N., Nezhat, C.N., Kempson, R., Lessey, B.A., Nayak, N.R., Giudice, L.C., 2006. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. Endocrinology 147, 1097–1121.

Tawfeek, M.A., Eid, M.A., Hasan, A.M., Mostafa, M., El-Serogy, H.A., 2012. Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility. BMC Womens Health 12, 10.

Thelen, J.J., Miernyk, J.A., 2012. The proteomic future: where mass spectrometry should be taking us. Biochem. J. 444, 169–181.

Tseng, L.H., Chen, I., Chen, M.Y., Yan, H., Wang, C.N., Lee, C.L., 2010. Genome-based expression profiling as a single standardized microarray platform for the diagnosis of endometrial disorder: an array of 126-gene model. Fertil. Steril. 94, 114–119.

van der Gaast, M.H., Beier-Hellwig, K., Fauser, B.C., Beier, H.M., Macklon, N.S., 2003. Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates. Reprod. Biomed. Online 7, 105–109.

Venetis, C.A., Kolibianakis, E.M., Papanikolaou, E., Bontis, J., Devroey, P., Tarlatzis, B.C., 2007. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in in vitro fertil-

ization? A systematic review and meta-analysis. Hum. Reprod. Update 13, 343–355.
Vilella, F., Ramirez, L.B., Simon, C., 2013. Lipidomics as an emerging tool to predict endometrial receptivity. Fertil. Steril. 99, 1100–1106.
Walsh, C.T., Garneau-Tsodikova, S., Gatto Jr., G.J., 2005. Protein posttranslational modifications: the chemistry of proteome diversifications. Angew. Chem. Int. Ed. Engl. 44, 7342–7372.
Wang, H., Dey, S.K., 2005. Lipid signaling in embryo implantation. Prostaglandins Other Lipid Mediat. 77, 84–102.
Young, S.L., 2013. Oestrogen and progesterone action on endometrium: a translational approach to understanding embryo receptivity. Reprod. Biomed. Online.
Zhang, Z., 2012. An in vitro diagnostic multivariate index assay (IVDMIA) for ovarian cancer: harvesting the power of multiple biomarkers. Rev. Obstet. Gynecol. 5, 35–41.

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