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Early Pregnancy Screening for Complications of Pregnancy: Proteomic Profiling Approaches

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1. Introduction

The keystone to improving health outcomes remains the timely and accurate diagnosis of the predisposition to, or early detection of, disease. Early detection of disease risk and onset is the first step in implementing efficacious treatment and improving patient outcome. (Figure 1). In the context of antenatal screening, the objective of proteomic approaches is to identify proteins and peptides that are informative of the risk of asymptomatic early pregnant women subsequently developing complications of pregnancy. That is, how the antecedents of complications of pregnancy alter the expression of the genome and how this is manifested as altered protein and peptide expression. Informative proteins and peptides identified may be used to develop classification models (*e.g.* multiple biomarker diagnostic

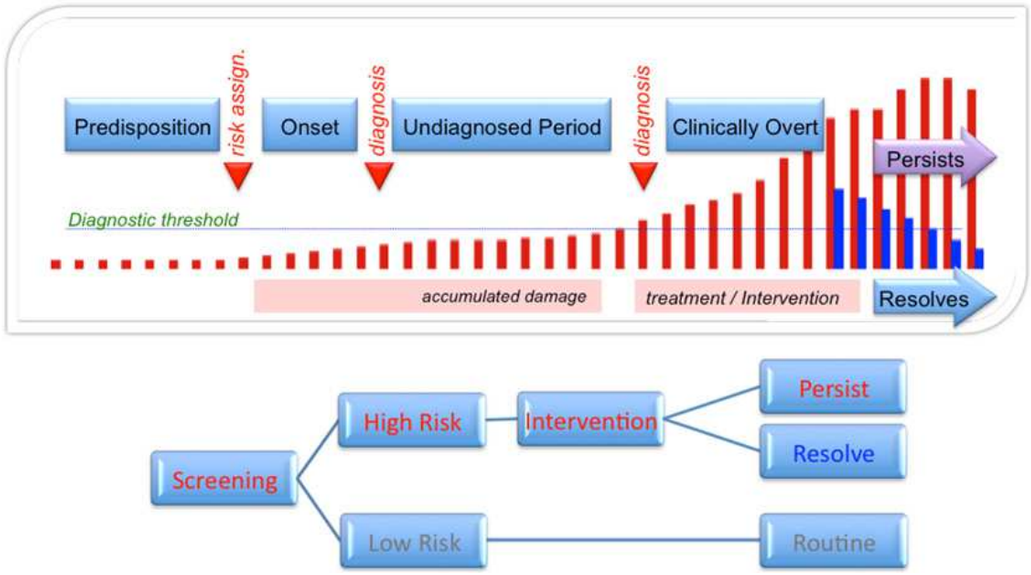

Fig. 1. The putative benefit of early pregnancy screening. A theoretical profile of disease progression in which disease onset is determined by diagnostic threshold. Once diagnosed, the condition can be treated and either persists or resolves. The rationale for both early screening and assessment of disease risk is early diagnosis of disease. Early diagnosis of disease affords the opportunity for early treatment and reduced adverse effects.

or prognostic tests) that assign the likelihood that an individual test sample came from a normal or "at risk" group. Such tests (as with all in vitro diagnostics, IVDs) inform clinical decision-making and provide an opportunity for timely and appropriate intervention. The performance of the test determines the quality of the information provided and ultimately the course of patient management. The bailiwick of proteomics, thus, extends beyond simply establishing the protein complement of a given sample and includes its contribution to the healthcare system.

Proteomic profiling technologies have undergone rapid development and diversification over the past decade, however, issues relating to the analysis of complex biological samples (such as plasma), achieving biomolecular bandwidth (*i.e.* the coverage of a given proteome that any one technique can attain) and translating outcomes into clinical practice remain (Rice *et al*, 2006). The objective of this brief commentary is to provide a conceptual and applications-based overview of how proteomic technologies may contribute to the development of IVDs for assigning risk of disease in both symptomatic and asymptomatic patients. At this time, there have been few Phase 2 (Pepe *et al*, 2001) (retrospective case control cohorts) and Phase 3 biomarker trials (longitudinal, cohort studies) completed that target the early pregnancy period (*i.e.* 6-12 weeks of gestation) and even fewer that consider complications other than chromosomal abnormalities or pre-eclampsia (PE).

2. Complications of pregnancy

Of the 130 million babies born each year, 8 million die before their first birthday. Four million babies die in the first 4 weeks of life (during the neonatal period). Three million of neonatal deaths occur in the first week, with the highest risk of death on the first day of life. More than 7 newborn babies die every second from what are ostensibly preventable causes (Zupan *et al*, 2005),(Lawn *et al*, 2005). A significant contributing factor in many of these deaths is poor pregnancy outcome as a result of a complication of pregnancy. Pre-eclampsia, intrauterine growth restriction (IUGR), gestational diabetes (GDM) and preterm birth (PTB) are the most important complications of pregnancy that have no effective antenatal treatment other than steroid administration and timely delivery. Each occurs with an incidence of 5-10% and are responsible for the majority of obstetric and paediatric morbidity and mortality and can permanently impact on life-long health. For example, PTB alone accounts for up to 2.7 million deaths per annum and ~50% of long-term neurological impairment. While, pre-eclampsia accounts for 10-15% of the 500,000 maternal deaths each year (Khan *et al*, 2006).

These complications of pregnancy are not usually clinically manifested until third trimester (*i.e.* > 24 weeks of pregnancy) thus limiting the window of opportunity to ameliorate adverse effects. Currently, there are no proven means of identifying asymptomatic women during the first trimester who subsequently develop complications of pregnancy (other than past obstetric history). Early detection of women at risk of complications of pregnancy would afford opportunity to develop and evaluate timely and appropriate intervention strategies to limit acute adverse sequelae (Figure 2).

The clinical imperative for the development of biomarkers for screening and monitoring pregnancy derives from the significant impact that undiagnosed, untreated and/or late-treated complications of pregnancy have on both the wellbeing of the mother and the newborn (including perinatal, neonatal and childhood development and adult susceptibility

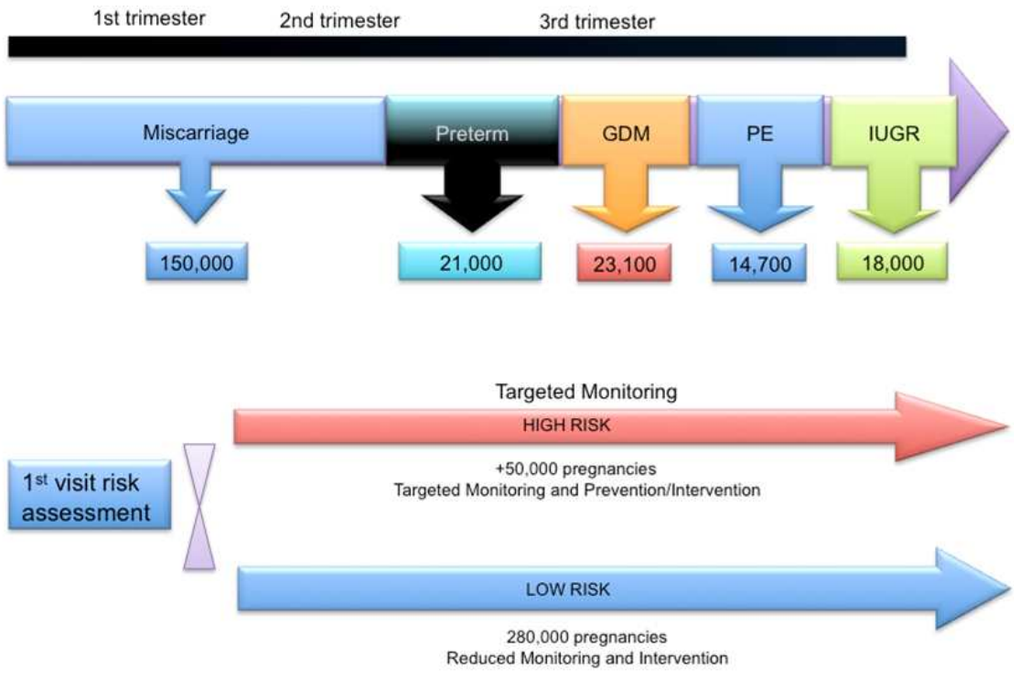

Fig. 2. In Australia in 2008, there were 294,700 live births (Laws *et al*, 2010). More than 60,000 women gave birth associated with a complication of pregnancy. 21,000 babies were born preterm (*i.e.* < 37 completed weeks of gestation). 18,000 babies were of low birthweight (<2500g). 23,100 pregnancies were complicated by GDM and 14,700 developed PE. Assessment of risk of developing a complication of pregnancy at first antenatal visit would provide opportunity to triage women to high- and low-risk management.

to disease). The development of predictive and diagnostic utilities for use at the first antenatal visit would provide, at least, an opportunity for more intensive monitoring of high-risk women and, at best, implementation of appropriate interventions.

Complications of pregnancy may represent symptom-defined manifestations of a single lesion. The available evidence supports the hypothesis that the etiology of complications of pregnancy (including for example PE, IUGR) may begin during 1st trimester (Brosens *et al*, 2002); (Meekins *et al*, 1994); (Jauniaux *et al*, 2006); (Norwitz, 2006). Such complications may originate from aberrant or suboptimal implantation and/or sequestration of the maternal uterine blood vessels. If pregnancy complications share a common etiology or elicit a common maternal response then changes in the profile (or specific patterns) of plasma proteins measured during early and /or mid-pregnancy may be informative in identifying women at risk. Identification of such proteins would provide opportunity to develop clinically useful early pregnancy screening tests to identify women at risk of developing complications during pregnancy. If this can be achieved it would provide an opportunity for early identification of risk and the implementation of an alternative clinical management to improve outcome for both mother and baby.

In addition to the acute effects on maternal and newborn morbidity and mortality, complications of pregnancy may adversely affect life-long disease susceptibility of the newborn and intergenerational health via epigenetic modification of the fetal genome (Weaver

et al, 2004; WHO, 2006). Epigenetic modification is defined as alteration of the regulation of genomic information by means that do not result in a change in DNA sequence, but have a significant impact on the development and phenotype of an organism (Santos & Dean, 2004). The epigenome is responsive to external environmental factors including, but not limited to, nutrition and endocrine disruptors. Epimutations in the germline that become permanently programmed may be transmitted as epigenetic transgenerational phenotypes. The "external" environment for the placenta (and fetus) is maternal blood. The placenta and fetal membranes play a critical role in filtering or buffering environmental influences (Myatt, 2006). Changes in the external *milieu* (*e.g.* blood pressure, hyperglycemia (Brasacchio *et al*, 2009); (El-Osta *et al*, 2008); ischemia (Kumral *et al*, 2009; Parker *et al*, 2008)) and/or diet (e.g. butyrate (Vidali *et al*, 1978), organosulfur (Tissenbaum & Guarente, 2001), dietary polyphenols (Howitz *et al*, 2003), folate, and choline (Fang & Xiao, 2003)) may induce adaptive or compensatory epigenetic modifications within the placental and/or fetal genomes. Thus, periconceptional and early pregnancy events may affect the placental and/or fetal epigenome. This may be particularly relevant for women who experience complications of pregnancy that impact on placental structure and function. Early detection of women at risk of complications of pregnancy would afford opportunity to develop and evaluate timely and appropriate intervention strategies to limit long-term and intergenerational adverse sequelae.

The rationale for developing antenatal screening tests, thus, is not only for the management of the contemporaneous pregnancy but also to optimise life-long and intergenerational health. The diagnostic performance of antenatal screening tests may not need to be high to be effective. Unlike diseases such as cancer where IVDs need to be exquisitely specific (Edgell *et al*, 2010a; Edgell *et al*, 2010b; Rice *et al*, 2010), a useful antenatal screening test would ideally be highly sensitive, but not necessarily highly specific. The consequence of a false positive would be no worse than an erroneous triage to high-risk care.

3. Early pregnancy screening

Previous approaches to develop an early pregnancy-screening test for women at risk of developing complications of pregnancy have not been of great success. For example, with respect to pre-eclampsia, while it has been possible to identify blood-borne biomarkers that have pre-symptomatic predictive potential (including: activin-A (Diesch *et al*, 2006), C-reactive protein (Mihu *et al*, 2008), placenta growth factor and its receptor FLT (Shokry *et al*), leptin (Sucak *et al*), transforming growth factor-β1 and plasminogen activator inhibitor (Belo *et al*, 2002)), such markers have proven of limited clinical utility, lacking suitable sensitivity and specificity. No single marker has been described permitting early prediction of pre-eclampsia in the individual.

It is now widely acknowledged that single biomarkers are unlikely to deliver significant incremental gain in sensitivity and specificity that will be required for the development of effective screening and classification tests requisite for the implementation of personalized medicine. New approaches based upon the measurement of multiple biomarkers of disease risk afford opportunity to increase diagnostic test sensitivity and specificity. Even the use of two biomarkers can deliver improved performance. For example, cardiovascular risk classification is significantly improved by simply combining LDL-cholesterol and C-reactive protein data (Rifai & Ridker, 2003). The use of modelling algorithms to combine multiple known biomarkers (*e.g.* candidate-based approaches) similarly may increase diagnostic

efficiency and deliver classification models of clinical utility (Fushiki *et al*, 2006; Kikuchi & Carbone, 2007; Kikuchi *et al*, 1989; Listgarten & Emili, 2005). Both candidate-based applications (*i.e.* in which the identity of the analytes being measured are well-established) and signature profiling applications (*i.e.* in which characteristic patterns or motifs within a signal profile are identified) may be utilized in the development of multivariate modelling strategies for the delivery of more informative diagnostic tests (Anderson, 2005).

Recent advances in the acquisition of proteomic and peptidomic data, methods of analysis and multivariate modelling approaches afford opportunities to deliver IVDs with increased diagnostic efficiencies. In the case of higher prevalence diseases, such as complications of pregnancy, these new approaches may contribute to the development of "first-visit" antenatal screening programs.

Using a similar rationale, (Horgan *et al*) recently reported that metabolomic profiles identified using ultra performance liquid chromatography-mass spectrometry were able to characterize a phenotypic signature for small for gestational age (SGA) babies. The authors concluded that the finding of a consistent discriminatory metabolite signature in early pregnancy plasma preceding the onset of SGA offers insight into disease pathogenesis and offers the promise of a robust pre-symptomatic screening test.

4. Defining the proteome

The proteome is the manifestation of the conditional expression of the genome. Proteomics, thus, defines the regional and temporal expression of proteins (and peptides) that characterize a given phenotype and how changes in expression impact the structure and function of the organism. It is the systematic, reproducible, differential and/or quantitative characterization of the peptide or protein complement under a defined biological state(s). In particular, its *raison d'être* is to elucidate networks and pathways that ensure coordinated and appropriate development of biologic organisms and to maintain homeostasis in response to physiological or pathological challenges. In its simplest form, proteomics is a reductive approach that reduces system complexity to more basic components, thus, enabling classical hypothesis testing. It affords the opportunity to characterize physiology and pathophysiology in terms of defined and specific changes in proteins and peptides that comprise the human proteome.

In recent years, it has been recognized that the complexity of the mammalian transcriptome and its functional expression as proteins far exceeds previous expectations. It is now estimated that only ~1.2% of the human genome contains protein-coding information. The expression of these ~21,000 genes, the elaboration of ~10^5 transcripts (*via* alternative splicing, alternate promoters and RNA editing) and the post-translational modification account for more than 10^6 proteins comprising the human proteome. It has been estimated, in some cases, that up to 100 different proteins may be derived from the expression of a single gene. An informed understanding of the ontogeny and complications of pregnancy, therefore, will include not only genomic and transcriptomic analysis but also information as to how global protein expression changes. This is the bailiwick of proteomics – defining the conditional expression of the genome**.**

As alluded to above, proteomic methodologies, however, now extend beyond the mapping of the protein complement of defined proteomes to proteome-wide profiling approaches

(Patterson & Aebersold, 2003). New approaches offer opportunities to: define protein expression profiles that reflect phenotypic change; and contribute to the development of prognostic and diagnostic applications. Such approaches apply preceptive filters to the proteome (*e.g.* knowledge-data bases, in the case of targeted proteomics or multivariate mathematical modelling in the case of protein/peptide profiling strategies) to extract data of contextual relevance. Proteomics affords opportunity to identify changes in specific subsets of proteins that are associated with variance from normal and to interrogate their role in the etiology of pregnancy complications.

There are three common approaches to the application of proteomics: cartographic or expression proteomics - the definition of normal expression profiles of proteins and peptides, how they are modified and processed (Di Quinzio *et al*, 2007); comparative profiling – in which protein expression profiles from different physiologic and/or pathologic states are compared for the purpose of identifying condition- or treatment-associated changes (Di Quinzio *et al*, 2008) and targeted profiling - in which specific known subsets of proteins are monitored (Heng *et al*, 2009) (Georgiou *et al*, 2008).

Common to the successful application of all approaches is the capacity to reduce sample complexity and to target a subfraction of the proteome for analysis, as no currently available platform provides proteome-wide display (Ahmed & Rice, 2005). With respect to sample complexity, a major challenge has been the identification of low abundance proteins that may reflect biological and/or clinical circumstance in the presence of overwhelming concentrations of high abundance protein species. Both depletion and enrichment sub-fractionation approaches have been employed with varying degrees of success, including affinity depletion of albumin (Ahmed *et al*, 2003), other high abundance proteins (Georgiou *et al*, 2001)) and affinity-capture enrichment of low abundance species (Callesen *et al*, 2009). It is becoming increasingly evident that combinations of multiple approaches and targeting of specific display bandwidths will be required to achieve the display resolution required to identify putative low abundance biomarkers.

5. Proteomic approaches for profiling early pregnancy

The identification of protein and peptide signatures or motifs contained within biological samples for the purpose of donor classification is a burgeoning area within the domain of diagnostic and predictive medicine. The premise upon which such initiatives are based is that: the expression of specific proteins or peptides and/or their metabolites is altered by and reflective or informative of the attendant pathophysiology; and the measurement and combination of multiple biomarkers of disease, may increase diagnostic sensitivity and specificity. Once established, reference profiles measured from healthy sample cohorts may be used as a template to detect variance and thus deliver a diagnostic or predictive capacity. Several proteomic-based approaches have been applied to identify informative biomarkers of complications of pregnancy, including protein solution array, 1 and 2 dimensional gel electrophoresis and mass spectrometry-based peptide profiling.

5.1 Solution array

Multiplex protein solution array has a number of advantages over current analyte quantification technologies, including: measurement of many biomarkers (theoretically, up

to 100 different analytes) in a single sample; wider operational dynamic range; and increased sensitivity and specificity derived from multivariate modelling of combinations of biomarker analytes. This system utilizes a sandwich ELISA-like protocol, in which capture antibodies are coupled to spectrally distinct beads. Biotinylated sandwich antibody and streptavidin- phycoerytherin fluorophore are used as a reporter complex. Bead identity and analyte-specific fluorescence are assessed using a flow cytometer.

Georgiou *et al*, 2008 utilized protein solution array to measure multiple plasma biomarkers at 11 weeks of gestation in women who subsequently experienced normal pregnancy outcomes and women who subsequently developed gestational diabetes. Of the biomarkers considered, three biomarkers (adiponectin, insulin and blood glucose) displayed informative diagnostic characteristics (*i.e.* area under the receiver operator characteristic curve, AUC, adiponectin =0.867; insulin =0.872 and glucose =0.827). When these markers were combined in a multivariate classification and predicted posterior probability values generated, the classification model generated significantly outperformed individual biomarkers (model AUC = 0.94). This simple example demonstrates the putative benefit of a multimarker approach for improving diagnostic efficiency. While this Phase 1 biomarker trial delivered promising data, a much larger trial is required to establish diagnostic performance in an obstetric population.

5.2 Gel-based profiling

The gel-based platforms such as 1-dimensional and 2-dimensional polyacrylamide gel electrophoresis (2D PAGE) and fluorescence 2D difference gel electrophoresis (DIGE) have been used in both expression and comparative studies to define plasma protein abundance and disease-associated or treatment-induced changes. The advantage of these approaches resides in their ability to identify post-translational modified protein isoforms. The limitation of gel-based systems is their relatively low throughput, the necessity for sample processing and fractionation prior to display and limited mass range (~10-200 kDa). In addition, procedural protein losses and the overall experimental variation in estimating endpoints by 2D PAGE may be considerable. Procedural losses of proteins during 2DE PAGE display have been reported to be as high as 80% but this can vary depending on the initial protein load. As with any other technique, variation is apportioned between technical replication, both within assay and between assay, and biologic variation (*i.e.* sample-to-sample). Estimates of the variation attributable to technical replication average 25-40%. Biological variation has been estimated to be between 24 and 70% (Molloy *et al*, 2003).

We have utilized a 2D-PAGE approach to identify putative plasma biomarkers of GDM. Using a traditional 2D PAGE approach, maternal plasma proteome from women with a normal pregnancy were compared with women who subsequently developed GDM. Using this approach more than 600 protein spots were visualized. Of these more than 20 proteins were differentially-expressed in pre-symptomatic women. Some of these protein spots are unique to pre-GDM while others are also differentially-expressed during overt disease. In some cases only specific isomers of a particular protein were differentially-expressed (Figure 3).

Using this approach, gestation-associated changes in plasma protein expression changes can be established for individual patients (Figure 4). This latter application may be of utility in the development of personalized medicine approaches to risk assessment and disease monitoring during pregnancy.

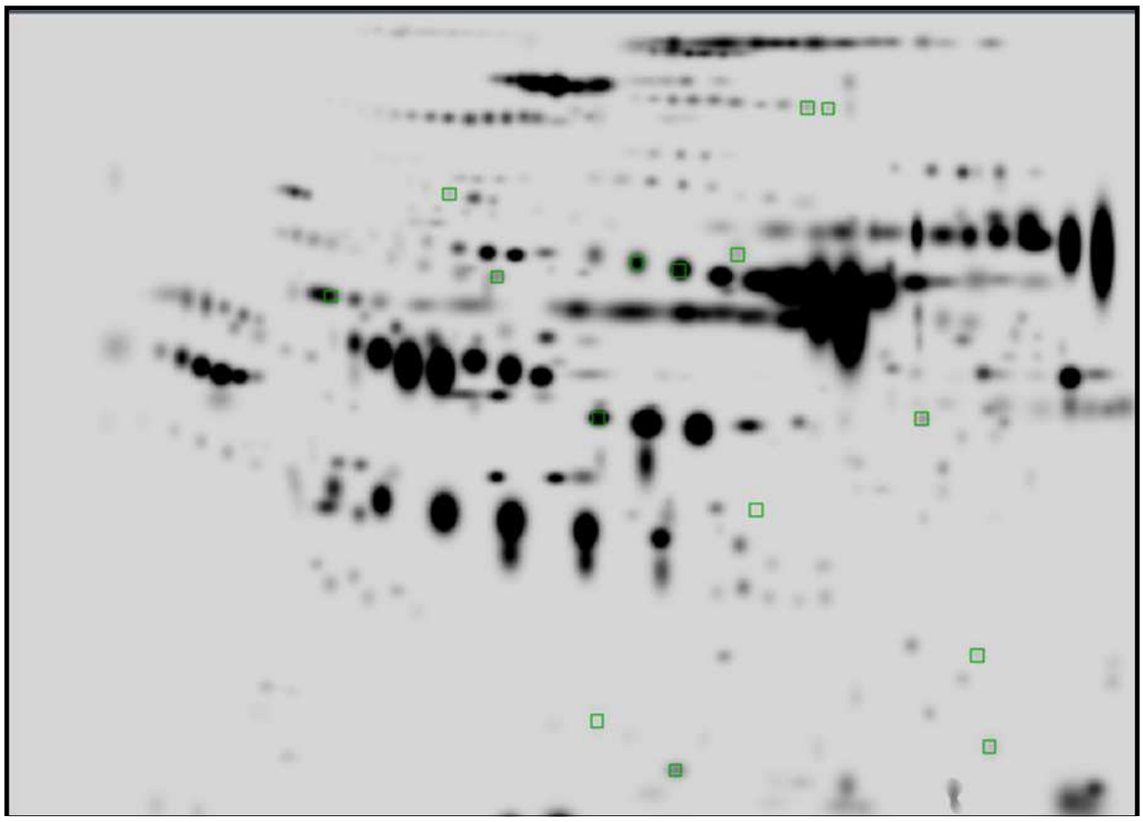

Fig. 3. 2D-PAGE Gaussian image of human plasma obtained at approximately 12 weeks' gestation. Boxes indicate protein spots that were significantly differentially-expressed in women who subsequently developed GDM compared to gestation-matched women who had a normal pregnancy.

The limitations of this methodology include (i) tedious and sometimes unreliable matching of hundreds of spots in multiple gels, (ii) problems associated with spot normalization, (iii) limited in-built statistical capacity to compare protein abundance, (iv) difficulty with excision of spots especially in small gel formats, and (v) the failure to reliably characterize proteins by MALDI-ToF mass spectrometry due to low protein abundance. This necessitates the need to up-scale methods for protein characterization (orthogonal identification).

Some of the limitations of gel-based approaches have been overcome with the development of difference gel electrophoresis. This minimal labeling approach using fluorescent cyanine dyes increases throughput by reducing sample processing and both gel-to-gel and analytical variation by combining case and control samples into a single processing step, and by the use of an internal standard for normalization of data across gels (as described above). DIGE also delivers useful relative quantification of protein expression profiles where the dyes are purported to have sub-nanogram sensitivity and a linear response to protein concentrations of over five orders of magnitude. The dyes are also compatible with mass spectrometric analysis. With respect to analyzing the plasma proteome, DIGE is still limited by the compositional complexity of plasma and similarly benefits from sample fractionation and the removal of high-abundance proteins.

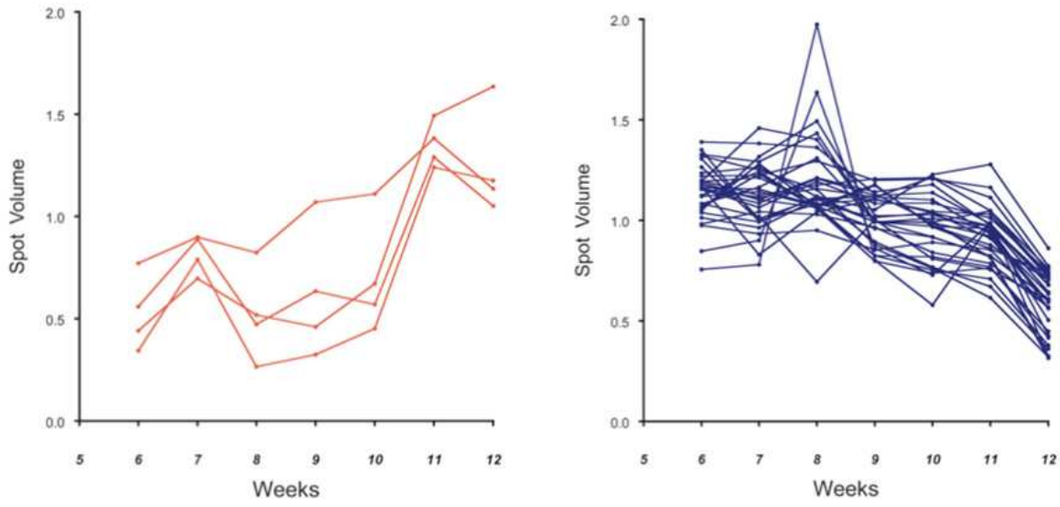

Fig. 4. Variation in plasma proteins displayed during early pregnancy (6-12 weeks of gestation). Serial peripheral blood samples were collected from women and displayed using 2D-DIGE. The data presented represent normalized spot volumes for protein spots identified that either increased or decreased during early gestation.

5.3 Mass spectrometry-based profiling

Mass spectrometry-based approaches for identifying and establishing relative changes in biomarker abundance included: stable isotope labeling techniques and label-free strategies. Stable isotope labeling has the advantage of being more sensitive and reproducible than gel-based methods. These approaches utilize either a mass tag coding strategy (*e.g.* ICPL - Isotope Coded Protein Labeling, ICAT - Isotope Coded Affinity Tag) or iTRAQ - isobaric Tag for Relative and Absolute Quantitation) that allow pooling of samples to reduce technical variation. Label-free quantitation is an approach that holds the promise of true MudPIT-type 'shotgun' quantitation and has some advantages in sample preparation, cost and the challenge of normalizing the data so that accurate quantitation can be done across multiple samples and multiple analyses.

In addition to its analytical applications, mass spectrometry affords opportunities to identify signature profiles contained within biological samples for the purpose of classification. The application of mass spectrometry is a burgeoning area within the domain of diagnostic and predictive medicine. This approach now affords the opportunity to develop disease-specific patterns or profiles based upon the presence of specific peptides in a patient sample. MS-based protein profiling relies on the presence and spatial relationships between peptide peaks to facilitate the classification of biological samples into different categories (*e.g.* normal and disease). Based upon the analysis of a training sample set (*e.g.* disease-free patients), pattern recognition software and multivariate modelling are employed to build peptide profiles or motifs that characterize a disease-free condition. Once established, such reference profiles may be used as a template to detect variance and thus deliver a diagnosis or predictive capacity.

Over the past 5 years, our research groups have utilized two mass spectrometry-based profiling approaches to identify peptides that may be informative of disease risk: affinity-

capture MALDI-ToF and iTRAQ. In an initial prospective study of plasma samples collected from women (at 6-12 weeks' and 26-30 weeks' gestation), samples were analyzed after removal of high abundance proteins and following a single fractionation process. Immobilized Metal Affinity Chromatography (IMAC, ClinProt™), Bruker Daltonics) was used to capture a subpopulation of peptides for subsequent MALDI-ToF mass spectrometry profiling. Complication-specific, differentially-expressed peptide ion peaks were identified (*e.g.* Figure 5) that provided classification models of promising utility.

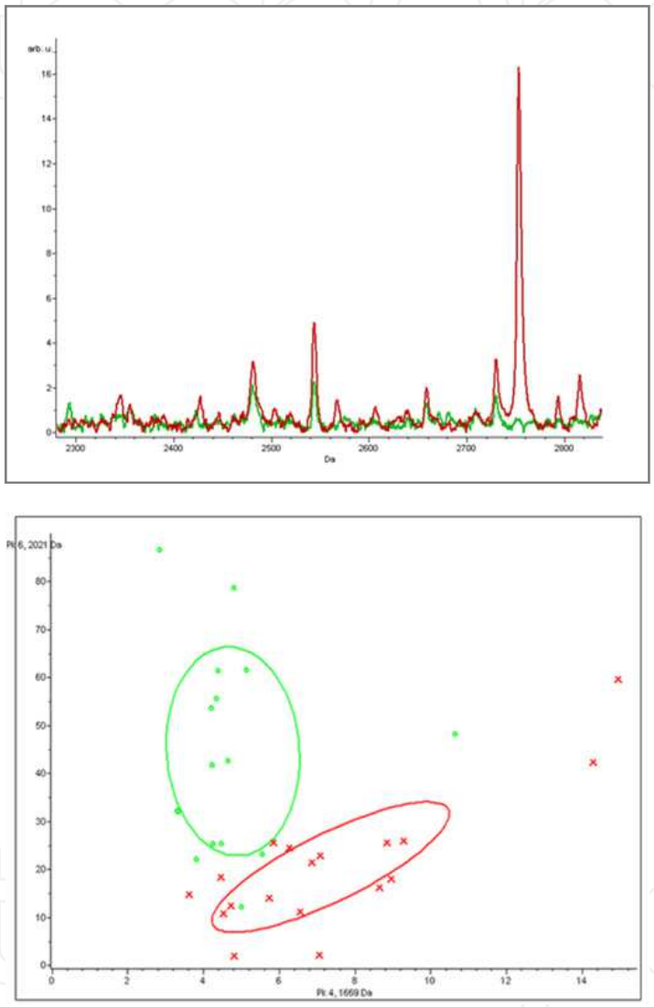

Fig. 5. An example of MALDI-ToF peptide profiling and bivariate cluster analysis. **Top.** Example of the average peptide profiles over a limited spectral range (2300-2800 m/z) is presented to illustrate identified differences in peptide profiles between women with a normal pregnancy (red, n=19, 12 weeks) and women who subsequently developed GDM (green, n=16, 12 weeks). **Bottom.** A peptide peak cluster plot highlighting the potential for using differentially-expressed peptides to classify women into low- and high-risk groups for subsequent development of GDM. The plot presents the data (integrated area) of two peptide peaks (1669 vs 2021 m/z) observed in plasma obtained from women (12 weeks' gestation) who subsequently experienced a normal (red) or GDM pregnancy (green). Standard deviation envelopes are presented.

Both bivariate cluster plots and multivariate modelling discriminated between women who subsequently experienced normal or complicated pregnancies. Disease-specific differentially-detected peptide ion peaks were identified and used to develop multivariate classification models (Support Vector Machine and Genetic Mutation Models) that discriminated between women who subsequently experienced a normal or GDM pregnancy. For example, using a genetic mutation classification model, 5 peptides were selected that had the ability to correctly classify 100% of women to a low-risk group (i.e. those women who subsequently experienced a normal pregnancy). Furthermore, the model correctly classified greater than 93% of those women who subsequently experienced a GDM pregnancy. While this Phase 1 biomarker trial has delivered promising data a further larger trial is required to validate these findings.

Of considerable note, was the observation that when data were combined into a normal and complicated group (*i.e.* all complications), both cluster analysis (Figure 6) and modelling algorithms of diagnostic utility were generated. This approach may be of use in the development of personalized medicine approaches to assess disease risk during pregnancy.

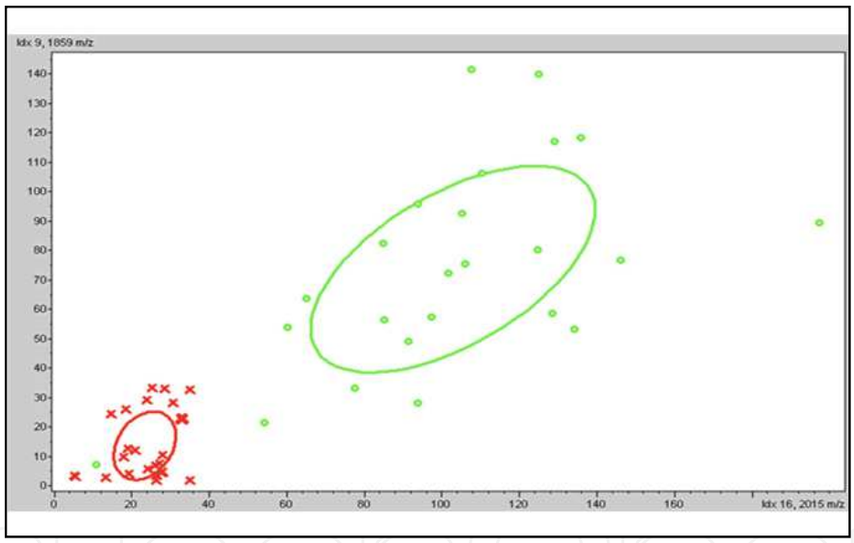

Fig. 6. A peptide peak cluster plot highlighting the potential for using differentially-expressed peptides to classify women into low- and high-risk groups for subsequent complication of pregnancy (PE, IUGR, GDM and PTB) . The plot presents the data (integrated area) of two peptide peaks (1859 vs 2015 m/z) observed in plasma. The plot depicts data obtained from women who subsequently experienced a normal (red) or experienced a complicated pregnancy (green). Standard deviation envelopes are presented.

Additionally, an iTRAQ mass spectrometry-based approach has been used to identify and quantify (relative to control) disease-specific peptide ion abundance in maternal plasma samples. This isotopic labeling method is arguably the benchmark for relative protein quantification. One significant benefit is that it allows sample multiplexing. High-abundance protein depleted plasma pools were generated from asymptomatic pregnant

women at 12-18 weeks' gestation. Samples were digested with trypsin and each digested sample was labeled with one of four different iTRAQ reagents (normal 114, IUGR 116, GDM 118 and Macrosomia 121) and analyzed by LC-MS/MS for simultaneous protein identification and peptide quantification. Relative abundance of proteins in depleted plasma was determined by comparing the peak heights of reporter ions. Using this approach, 22 proteins that were differentially-expressed in maternal plasma in association with complications of pregnancy were unambiguously identified. Further studies are currently assessing the performance of these biomarkers in IVD panels.

6. Signature profiling and IVD development

A recent trend in the development of more efficient diagnostic tests has been the use of algorithm-based multivariate index assays (IVDMIAs). With the development of this new class of IVD, the discipline has sought new biostatistical approaches for assessing and quantifying incremental gains in diagnostic efficiency. Traditionally, the area under the receiver operator characteristic curve (AUC) has been used as a measure and comparator of diagnostic efficiency. Several investigators have argued that this measure alone may be imperfect and inefficient for comparing the true clinical usefulness of alternative marker panels (Pencina *et al*; Pencina *et al*, 2008). These authors reviewed several biomarker studies and observed that when evaluating improvement in risk assignment of biomarkers, very large odds ratios were often associated with very small increases in the AUC. This feature of the receiver operator characteristic curve analysis limits its utility in identifying putative beneficial contributions of new biomarkers to algorithm-based models. Pencina *et al*, therefore, proposed the use of two new methods for evaluating the diagnostic efficiency of biomarkers. These two methods are: (i) Net Reclassification Improvement (NRI); and (ii) Integrated Discrimination Improvement (IDI). The NRI is based on counts of the number of true positives showing an increase in probability of an event and the number of true negatives showing a decrease in probability of an event. The IDI is based on the integral of sensitivity and specificity of all possible thresholds. These new methods provide alternative statistical approaches for validating biomarkers and IVDMIAs.

7. Concluding comments

Complications of pregnancy remain a major health issue of the 21st century. They result in preventable mortality and morbidity to both mother and baby. Too few studies have focused on the development of early pregnancy risk assessment modalities. As a consequence, it has not been possible to robustly evaluate any putative early pregnancy intervention strategies that may improve pregnancy and life-long outcomes. Indeed, driven by health economic imperatives, it is becoming increasingly more difficult to establish longitudinal early pregnancy study cohorts (*i.e.* from 6 weeks of pregnancy) in our tertiary care hospitals. With recent advances in proteomic and peptidomic technologies and the development of formalized approaches for establishing and validating multivariate index assays, it is not unrealistic to suggest that more informative antenatal screening tests will be implemented within the next 5-10 years. Such IVDs would allow, at least, the triage of women at 6-12 weeks of pregnancy into high- and low-risk clinical management pathways.

Furthermore, we recognize the imperative to establish not only early pregnancy clinics but also preconception clinics to ensure an optimal start to life and lower risk of adult disease.

8. Acknowledgements

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9. References

Ahmed N, Barker G, Oliva K, Garfin D, Talmadge K, Georgiou H, Quinn M, Rice G (2003) An approach to remove albumin for the proteomic analysis of low abundance biomarkers in human serum. *Proteomics* 3: 1980-7

Ahmed N, Rice GE (2005) Strategies for revealing lower abundance proteins in two-dimensional protein maps. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences* 815: 39-50

Anderson L (2005) Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J Physiol (Lond)* 563: 23-60

Belo L, Santos-Silva A, Rumley A, Lowe G, Pereira-Leite L, Quintanilha A, Rebelo I (2002) Elevated tissue plasminogen activator as a potential marker of endothelial dysfunction in pre-eclampsia: correlation with proteinuria. *Bjog-an International Journal of Obstetrics and Gynaecology* 109: 1250-1255

Brasacchio D, Okabe J, Tikellis C, Balcerczyk A, George P, Baker EK, Calkin AC, Brownlee M, Cooper ME, El-Osta A (2009) Hyperglycemia Induces a Dynamic Cooperativity of Histone Methylase and Demethylase Enzymes Associated With Gene-Activating Epigenetic Marks That Coexist on the Lysine Tail. *Diabetes* 58: 1229-1236

Brosens JJ, Pijnenborg R, Brosens IA (2002) The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol* 187: 1416-23

Callesen AK, Madsen JS, Vach W, Kruse TA, Mogensen O, Jensen ON (2009) Serum protein profiling by solid phase extraction and mass spectrometry: A future diagnostics tool? *Proteomics* 9: 1428-1441

Di Quinzio MKW, Georgiou HM, Holdsworth-Carson SJ, Ayhan M, Heng YJ, Walker SP, Rice GE, Permezel M (2008) Proteomic analysis of human cervico-vaginal fluid displays differential protein expression in association with labor onset at term. *J Proteome Res* 7: 1916-1921

Di Quinzio MKW, Oliva K, Holdsworth SJ, Ayhan M, Walker SP, Rice GE, Georgiou HM, Permezel M (2007) Proteomic analysis and characterisation of human cervico-vaginal fluid proteins. *Aust N Z J Obstet Gynaecol* 47: 9-15

Diesch CH, Holzgreve W, Hahn S, Zhong XY (2006) Comparison of activin A and cell-free fetal DNA levels in maternal plasma from patients at high risk for preeclampsia. *Prenat Diagn* 26: 1267-1270

Edgell T, Martin-Roussety G, Barker G, Autelitano DJ, Allen D, Grant P, Rice GE (2010a) Phase II biomarker trial of a multimarker diagnostic for ovarian cancer. *J Cancer Res Clin Oncology* 3: 1079-89.

Edgell TA, Barraclough DL, Rajic A, Dhulia J, Lewis KJ, Armes JE, Barraclough R, Rudland PS, Rice GE, Autelitano DJ (2010b) Increased plasma concentrations of anterior gradent 2 protein are positively associated with ovarian cancer. *Clin Sci (Lond)* 118: 717-725

El-Osta A, Brasacchio D, Yao DC, Pocai A, Jones PL, Roeder RG, Cooper ME, Brownlee M (2008) Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 205: 2409-2417

Fang JY, Xiao SD (2003) Folic acid, polymorphism of methyl-group metabolism genes, and DNA methylation in relation to GI carcinogenesis. *J Gastroenterol* 38: 821-9

Fushiki T, Fujisawa H, Eguchi S (2006) Identification of biomarkers from mass spectrometry data using a "common" peak approach. *BMC Bioinformatics* 7: 358

Georgiou HM, Lappas M, Georgiou GM, Marita A, Bryant VJ, Hiscock R, Permezel M, Khalil Z, Rice GE (2008) Screening for biomarkers predictive of gestational diabetes mellitus. *Acta Diabetologica* 45: 157-165

Georgiou HM, Rice GE, Baker MS (2001) Proteomic analysis of human plasma: failure of centrifugal ultrafiltration to remove albumin and other high molecular weight proteins. *Proteomics* 1: 1503-6

Heng YJJ, Di Quinzio MKW, Permezel M, Rice GE, Georgiou HM (2009) Multiple Novel Predictors of Human Labor. *Reproductive Sciences* 16: 160

Horgan RP, Broadhurst DI, Walsh SK, Dunn WB, Brown M, Roberts CT, North RA, McCowan LM, Kell DB, Baker PN, Kenny LC Metabolic Profiling Uncovers a Phenotypic Signature of Small for Gestational Age in Early Pregnancy. *J Proteome Res* 10: 3660-3673

Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* 425: 191-6

Jauniaux E, Poston L, Burton GJ (2006) Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. *Hum Reprod Update* 12: 747-55

Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PFA (2006) WHO analysis of causes of maternal death: a systematic review. *Lancet* 367: 1066-1074

Kikuchi T, Carbone DP (2007) Proteomics analysis in lung cancer: challenges and opportunities. *Respirology* 12: 22-8

Kikuchi Y, Kita T, Iwano I, Kuki E, Oomori K, Kizawa I (1989) Prediction of clinical course and recurrence of human ovarian cancer by several serum tumor markers. *Nippon Sanka Fujinka Gakkai Zasshi - Acta Obstetrica et Gynaecologica Japonica* 41: 69-76

Kumral A, Tuzun F, Yesilirmak D, Duman N, Ozkan H (2009) Role of epigenetic regulatory mechanisms in neonatal hypoxic-ischemic brain injury. *Medical Hypotheses* 72: 692-693

Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival S (2005) Neonatal survival 1 - 4 million neonatal deaths: When? where? why? *Lancet* 365: 891-900

Laws P, Li Z, Sullivan EA (2010) Australia's mothers and babies 2008. Perinatal statistics series no. 24. Cat. no. PER 50. , AIHW. (ed). Canberra

Listgarten J, Emili A (2005) Statistical and computational methods for comparative proteomic profiling using liquid chromatography-tandem mass spectrometry. *Mol Cell Proteomics* 4: 419-434

Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR, van Asshe A (1994) A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol* 101

Mihu D, Costin N, Mihu CM, Blaga LD, Pop RB (2008) C-reactive protein, marker for evaluation of systemic inflammatory response in preeclampsia. *Rev Med Chir Soc Med Nat Iasi* 112: 1019-25

Molloy MP, Brzezinski EE, Hang J, McDowell MT, VanBogelen RA (2003) Overcoming technical variation and biological variation in quantitative proteomics. *Proteomics* 3: 1912-9

Myatt L (2006) Placental adaptive responses and fetal programming. *J Physiol* 572

Norwitz ER (2006) Defective implantation and placentation: laying the blueprint for pregnancy complications. *Reprod Biomed Online* 13: 591-599

Parker MD, Chambers PA, Lodge JPA, Pratt JR (2008) Ischemia-reperfusion Injury and Its Influence on the Epigenetic Modification of the Donor Kidney Genome. *Transplantation* 86: 1818-1823

Patterson SD, Aebersold RH (2003) Proteomics: the first decade and beyond. *Nat Genet* 33 Suppl: 311-23

Pencina MJ, D'Agostino RB, Steyerberg EW Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Statistics in Medicine* 30: 11-21

Pencina MJ, D'Agostino RB, Vasan RS (2008) Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. *Statistics in Medicine* 27: 157-172

Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y (2001) Phases of bimarker development for early detection of cancer. *J Natl Cancer Inst* 93: 1054-1056

Rice GE, Edgell T, Autelitano DJ (2010) Evaluation of midkine and anterior gradient 2 in a multimarker panel for the detection of ovarian cancer. *Journal of Experimental & Clinical Cancer Research* 29: 62

Rice GE, Georgiou HM, Ahmed N, Shi G, Kruppa G (2006) Translational proteomics: Developing a predictive capacity - A review. *Placenta* 27: S76-S86

Rifai N, Ridker PM (2003) Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. *Clin Chem* 49: 666-9

Santos F, Dean W (2004) Epigenetic reprogramming during early development in mammals. *Reproduction* 127: 643-651

Shokry M, Bedaiwy MA, Fathalla MMF, Alsemary A, Elwakil S, Murphy A Maternal serum placental growth factor and soluble fms-like tyrosine kinase 1 as early predictors of preeclampsia. *Acta Obstetricia Et Gynecologica Scandinavica* 89: 143-146

Sucak A, Kanat-Pektas M, Gungor T, Mollamahmutoglu L Leptin levels and antihypertensive treatment in preeclampsia. *Singapore Medical Journal* 51: 39-43

Tissenbaum HA, Guarente L (2001) Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* 410: 227-30

Vidali G, Boffa LC, Bradbury EM, Allfrey VG (1978) Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histones H3 and

H4 and increased DNase I sensitivity of the associated DNA sequences. *Proc Natl Acad Sci U S A* 75: 2239-43

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. *Nature neuroscience* 7: 847-54

WHO (2006) Promoting optimal fetal development: report of a technical consultation. *World Health Organization*

Zupan J, Aahman E, 2005 (2005) Perinatal mortality for the year 2000: estimates developed by WHO. Geneva: World Health Organization

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This book contains the total of 19 chapters, each of which is written by one or several experts in the corresponding field. The objective of this book is to provide a comprehensive and most updated overview of the human placenta, including current advances and future directions in the early detection, recognition, and management of placental abnormalities as well as the most common placental structure and functions, abnormalities, toxicology, infections, and pathologies. It also includes a highly controversial topic, therapeutic applications of the human placenta. A collection of articles presented by active investigators provides a clear update in the area of placental research for medical students, nurse practitioners, practicing clinicians, and biomedical researchers in the fields of obstetrics, pediatrics, family practice, genetics, and others who may be interested in human placentas.

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