## Abstracts

### *Alexander Bruce*

*Generating different genetic expression patterns*

The divergence of two differentiating extraembryonic cell types (trophectoderm and primitive endoderm) from the pluripotent epiblast population (a source of foetus progenitor cells) by the blastocyst stage of mouse development relies upon the activation and execution of lineage specific gene expression programs. Our understanding of the central transcription factor 'effectors' directing these cell-fate choices has accumulated rapidly. What is less clear is how the differential expression of such genes within the diverging lineages is initially generated? This review will summarise and consolidate our current understanding. I will introduce the traditional concept and importance of a cell's spatial location within the embryo, referencing recent mechanistic and molecular insights relating to cell-fate. Additionally, I will address the growing body of evidence which suggests that heterogeneities among blastomeres precede, and possibly inform, their spatial segregation in the embryo. I will also discuss whether the origins of such early heterogeneity are stochastic and/or indicative of intrinsic properties of the embryo. Lastly, I will argue that the robustness and regulative capacity of preimplantation embryonic developmental may reflect the existence of multiple converging, if not wholly redundant, mechanisms that act together to generate the necessary diversity of inter-cell lineage gene expression patterns.

### *Maria Elena Torres-Padilla*

*Generation of epigenotypes in embryonic development*

Embryonic development is a specificity of metazoans. It starts with the fertilisation of the oocyte by a sperm. Following fertilisation, the gametes undergo intense chromatin remodelling and epigenetic reprogramming, which is necessary to revert into a totipotent state, necessary to start a new developmental program. This reprogramming process must occur with 100% efficiency in order to sustain development. The period that follows fertilisation is therefore very rich and interesting in terms of chromatin remodelling. The latter underlies the reprogramming of the parental genomes, which is thought in turn to be essential to achieve the plasticity required to form all cell types in the new organism. However, this amazing capacity of the cells in the embryo to generate all cell types seems to be transient. Indeed, the ability of the early embryo to reprogram somatic nuclei decreases as development proceeds. What makes the cells in the early embryo capable of supporting such a large degree of plasticity ? These questions have remained largely unanswered and are central for our understanding of cell plasticity, development, and reprogramming. We propose that the basis of such plasticity relies on the distinctive chromatin features that prevail during early embryogenesis and will discuss this hypothesis in light of our recent findings.

### *Gijs Teklenburg*

*Embryo endometrial interactions in vitro*

Molecular interactions at the embryo-endometrial interface during the period of implantation are not fully understood. Our knowledge is primarily based on

available genetic and molecular evidence from implantation studies in the mouse. However, ethical and technical issues and the apparent differences between the various species, hinder our understanding of implantation in humans. Employing a human co-culture model, consisting of decidualizing endometrial stromal cells and single hatched blastocysts, we identified soluble factors involved in implantation. The endometrial component in the co-culture model acted as a biosensor of embryo quality upon differentiation into decidual cells. In view of the high incidence of gross chromosomal errors in human preimplantation embryos, cyclic decidualization followed by menstrual shedding may represent a mechanism of natural embryo selection that limits maternal investment in developmentally impaired pregnancies. Conversely, impaired decidualization predisposes to late implantation, negates embryo quality control, and causes early placental failure, regardless of the embryonic karyotype. These findings suggest a novel pathological pathway that unifies maternal and embryonic causes of recurrent pregnancy loss. Analysis of mid-secretory endometrial biopsies demonstrated that recurrent pregnancy loss is indeed associated with decreased expression of the decidual marker prolactin (PRL) but increased levels of prokineticin-1 (PROK1), a cytokine that promotes implantation.

***Jan J Brosens***

*Uterine memory and reproductive failure*

The invasive nature of human embryos and high incidence of aneuploidy necessitate a fail-safe mechanism to ensure prompt rejection of pregnancies that could threaten maternal wellbeing or survival. The most striking maternal innovation, confined to a handful of species, is cyclic decidualization (differentiation) of the endometrial stromal compartment, a process that couples uterine receptivity to menstrual shedding. Coordinated expression of decidual genes is hardwired by Eutherian transposons, acting as novel *cis*-regulatory elements that control specific gene networks in human endometrial stromal cells (HESCs). Cyclic menstruation imposes a need for continuous recruitment of mesemchymal stem-like cells that are programmed into mature HESCs responsive to decidual cues. Evidence of aberrant epigenetic programming of uterine cells is present in women with recurrent pregnancy loss (RPL). HESCs from RPL patients exhibit disease-specific epigenetic signatures and are poised to mount a distinct decidual response, characterized foremost by heightened expression of pro-inflammatory mediators. This in turn prolongs the expression of uterine receptivity genes and increases fertility. These reproductive gains, however, are offset by an increased risk of out-of-phase implantation and early pregnancy loss. The same epigenetic mechanisms underpinning cyclic decidualization render the human uterus responsive to -and adapted by - pregnancy failure; thus increasing the likelihood of subsequent reproductive success.

***Peter Parham***

*Pregnancy, immunology, and evolution*

Vertebrate systems of innate and adaptive immunity are more than 400 million years old, whereas mammalian placentation evolved 130 million years ago. Placentation evolved in the context of an immune system and has co-opted

immune system cells and molecules. Natural killer (NK) cells are lymphocytes that provide defence against viral infection and also co-operate with trophoblast on the invasive remodeling of maternal vessels that supply the placenta with blood. Controlling these processes are highly variable NK cell receptors that recognize polymorphic major histocompatibility complex (MHC) class I molecules as their ligands. As a consequence of strong and varying selection pressures, these systems of ligands and receptors evolve rapidly and are inherently unstable; thus they have been lost and reinvented on several occasions during mammalian evolution. The human system of killer cell immunoglobulin-like receptors (KIR) only has counterparts in monkeys and apes, species in which the co-evolution of receptors with ligands has been tracked. The emergence and diversification of MHC-C and its cognate KIR in hominids correlates with an increasingly invasive placenta. During human evolution the KIR system has undergone unique, qualitative changes that set it apart from the chimpanzee KIR system. The possible cause of these differences will be discussed.

***Y.M. Dennis Lo***

*Noninvasive prenatal diagnosis: from science to clinical applications*

Since 1997, it is known that cell-free fetal nucleic acid is present in the plasma of pregnant women. Early work has focused on the detection of paternally-inherited sequences that are not present in the mother's genome. Recent work on the use of digital PCR and massively parallel sequencing has facilitated the application of cell-free fetal DNA analysis to a number of single gene disorders and chromosomal aneuploidies, and has allowed fetal genome sequencing to be performed from maternal plasma. In addition to circulating fetal DNA, fetal RNA has also been detected in maternal plasma and has enabled non-invasive fetal gene expression profiling in a number of conditions, including pre-eclampsia. It is thus expected that non-invasive prenatal diagnosis using circulating fetal nucleic acids would likely replace a number of conventional prenatal testing modalities in the future. This technology could also be used as a safe confirmatory step in pregnancies involved in preimplantation diagnosis.

***Bert Smeets***

*Preventing the transmission of mitochondrial DNA disorders: selecting the good guys or kicking out the bad guys*

Mitochondrial disorders represent the most common group of inborn errors of metabolism. Clinical manifestations can be extremely variable, ranging from single affected tissues to multisystemic syndromes. In general, tissues with a high energy demand, like brain, heart and muscle, are affected. Maternally inherited mitochondrial DNA (mtDNA) mutations are a frequent cause, affecting ~1 in 5,000 individuals. The expression of mtDNA mutations differs from nuclear gene defects. Mutations are either homoplasmic or heteroplasmic and in the latter case disease become manifest when the mutation load exceeds a tissue-specific threshold. Mutation load can vary between tissues and in time, and often an exact correlation between mutation load and clinical manifestations is lacking. Because of the possible clinical severity, the lack of treatment and the high recurrence risk of affected offspring for carrier females, couples request to prevent transmission of mtDNA mutations. For many years, choices have been

limited, due to a segregational bottleneck, which makes the mtDNA mutation load in embryos highly variable and largely unpredictable. However, technically and ethically challenging possibilities, like preimplantation genetic diagnosis, pronuclear transfer and maternal spindle transfer, are becoming available or emerge at the horizon, providing carriers a new prospect of giving birth to a healthy child.

***Piraye Yurttas Beim***

*A report from the front lines of the personalized reproductive medicine revolution*

A short decade after the decoding of the human genome, whole genome sequencing is on the brink of becoming a bench-top technology akin to PCR. With the sub-$1000 dollar genome around the corner, significant challenges remain for translating the terabytes of data being generated in single experiments to breakthroughs in personalized medicine. The merging of private sector, clinical, and academic resources allows a number of these challenges to be more efficiently addressed. Our consortium has made significant progress on translating these new technologies to personalizing reproductive medicine. This progress includes the creation of a multi-center, de-identified clinical database and biorepository. It also includes the generation of accurate machine learning data analytical models for predicting outcomes of relevance for reproductive biology and medicine. A fertility-centric, genetic variant and functional annotation database has also been developed to address the limitations of commercially available databases, which are more optimal for cancer research. These tools are powering a predictive biomarker discovery platform that will make personalized reproductive biology and gynecology a reality in the next decade. The next set of challenges will be in how to best deliver and communicate this information to clinicians and patients to empower better clinical decision-making.

***Mellissa RW Mann***

*Assisted reproduction technologies and epigenetics*

Within the last 10 years, the efficacy and safety of human assisted reproductive technologies have come under intense questioning. While the absolute risks are low, accumulating evidence suggests that children conceived by assisted reproductive technologies are at an increased risk of developing genomic imprinting disorders. This may relate to the use of assisted reproductive technologies during crucial events that establish and maintain genomic imprinting during gametogenesis and earlier embryogenesis. To examine this question, we have investigated the effects of superovulation (drug-induced production of multiple eggs) and *in vitro* embryo culture on genomic imprinting in a mouse model system. Our data show that preimplantation development is a critical period for imprint regulation, that is susceptible to assisted reproductive technology-induced perturbations during *oocyte* and *preimplantation development*. More specifically, we have demonstrated that imprint maintenance is disrupted by superovulation and embryo culture. For superovulation, this disruption does not originate in the oocyte as a result of maternal imprint acquisition errors. Given that both technologies can lead to imprint maintenance errors, we explore the possibility that various assisted reproductive technologies may disrupt the same biological pathways that lead to disruptions in genomic

imprinting.

**Erica Watson**
*Transgenerational effects of folate on embryo and placenta development*
It is well known that maternal folate deficiency increases the risk of congenital abnormalities. Yet, little is understood about the molecular mechanism behind this phenomenon. To understand this further, we studied a genetic mouse model containing a mutation in a key gene (*Mtrr*) involved in folate metabolism. Remarkably, we observed that when either maternal grandparent was a carrier for this mutation, their wildtype grandprogeny was at risk for a wide spectrum of defects at midgestation (e.g., growth restriction, developmental delay and congenital abnormalities) even when the parents were wildtype. The folate cycle is necessary to transmit one-carbon methyl groups for the methylation of cellular components (e.g., DNA). We observed that the wildtype grandprogeny had genome-wide hypomethylation and locus-specific dysregulation of DNA methylation associated with the misexpression of developmentally important genes. Phenotypic analysis also indicated that some defects persisted up to four wildtype generations after the initial mutation suggesting a transgenerational effect of folate metabolism, which may involve the inheritance of epimutations. Ultimately, grandparental polymorphisms in folate metabolism may pose a risk and the benefits of folate fortification programs may take more than one generation to observe.

***Jonathan van Blerkom***
*Mitochondrial plasma membrane remodeling and developmental competence in the human*
The extent to which mitochondria have a determinant role in the normality of mammalian oogenesis and early embryogenesis has recently received considerable attention in model systems (mouse), commercially important species (bovine), and with respect to outcome, clinical IVF. While the notion that compromised mitochondrial function could directly influence outcome by adversely affecting oocyte fertilization and embryo competence has been generally accepted, assumed clinical relevance involves findings that are often conflicting, obtained from highly selected patient populations, or based on downstream lethality after exposure to chemical or environmental agents known to effect mitochondrial function and integrity. My presentation focuses on the importance of getting mitochondrial function in early human development 'right' in order to encourage investigation of novel methods to assess function noninvasively, and to better evaluate whether procedures suggested to improve outcome, such as nutritional supplementation to promote mitochondrial 'health,' or autologous mitochondrial transfer to 'rejuvenate' or 'refresh' the cytoplasm of the oocyte/early embryo are physiologically credible and clinically acceptable. In the context of Professor Edwards' interest in molecular polarity as a driving force in early development, the apparent importance for developmental competence of spatial compartmentalization of differential mitochondrial activity within the oocyte and early blastomere cytoplasm is emphasized.

***Marcos Meseguer***
*Is morphokinetic analysis the answer?*

Time-lapse observation presents an opportunity for optimizing embryo selection based on morphological grading as well as providing novel kinetic parameters, which may further improve accurate selection of viable embryos. We are presenting the largest set of transferred embryos after time-lapse analysis and thus a novel opportunity to correlate morphokinetic parameters to implantation and ongoing pregnancy. We have generated and evaluated a tool for the selection of viable embryos based on the exact timing of embryo developmental events together with morphological patterns by using an automatic time-lapse system to monitor embryo development. Several hierarchical and additive models are being developed.

We have elaborated a hypothesis, trying to elucidate whether time-lapse monitoring system together with embryo selection by morphokinetics is able to improve reproductive outcome. To undergo this objective we compared the result of an incubator with a built-in time-lapse video system (TMS) to our standard procedure involving normal incubators (SI) and sequential assessment by microscopy. The study employs logistic regression model to compare the clinical pregnancy for incubations in the TMS with incubations in a SI. Ultimately, several variables were evaluated as confounding factors (CF) that could possibly affect the outcome. The analysis revealed that TMS had a significant positive impact on chance of clinical pregnancy.

Our intention is to calculate and demonstrate the extent of the positive impact of TMS on pregnancy. Possible explanations for the observed increase could be : i) strictly controlled conditions, ii) reduced handling, iii) occasional observation of abnormal cleavages ; or iv) selection by morphokinetics related to embryo implantation. We are currently performing a RCT and an intermediate analysis will be presented.

***Peter Braude***

*Are the 'best' embryos being selected and what are the prospects for improvement?*

A successful outcome to an IVF cycle is a singleton healthy baby delivered at term. Sadly for most couples embarking on ART this a dream rather than a reality. In the absence of reliable methods for selecting a viable embryo in vitro, more than one embryo was replaced as the norm, which resulted in an epidemic of multiple births with its attendant hazards to mother and child. Even when a single embryo is replaced, most do not result in pregnancy, and when they do, nearly one in four successful implantations will be followed by miscarriage. Although some failures maybe the result of inadequate endometrial receptivity, a significant number may be due to developmental or structural errors within the embryo. Many attempts have been made to improve outcome by better selection of embryos during preimplantation development including a hunt for materials secreted within the culture medium (metabolomics), an examination of development patterns (morphokinetic), or genetic analysis of the embryos (cytogenetic and molecular) estimated from a biopsy hoped to represent the genetic constitution of the whole embryo. All such tests require a high degree of certainty since each method involves removing from the cohort that was available to be replaced, those deemed unfit. How do we select the 'best' embryo, and will this really improve IVF outcome?

***Roger Gosden***

*Programmes and prospects for ovotechnology*

As central players in reproduction, oocytes have enormous significance in biology, pathology and ageing. Since they are rare before menopause and extinct afterwards, boosting the ovarian reserve or creating them *de novo* in culture could provide oocytes for medical commerce in IVF and egg donation, cloning research, premature ovarian insufficiency, and perhaps even help to improve egg quality and extend the natural reproductive lifespan. Unlike the male, however, the female germ line is truncated at birth by differentiation at the onset of meiotic prophase, or so it was agreed almost universally until lately. This paradigm implies the oocyte store is finite and will become exhausted by follicular atresia and recruitment for ovulation, which, according to Henderson and Edwards, operates as a production line. But recently it was claimed that oogonia persisting after birth can potentially generate new follicles, which would revolutionize ovarian biology and technology. Other studies have reported oocyte-like cells emerging spontaneously in culture from pluripotent stem cells or even from somatic tissues. Do these claims predict a new road map, or can oocyte numbers only be increased by rescuing those already formed, as conventional theory prescribes?

***Daniel Brison***

*How should we assess safety of oocyte vitrification and other novel IVF technologies?*

Clinical IVF treatment was established more than 30 years ago via the pioneering work of Edwards and Steptoe and other teams around the world, and is now considered routine treatment. However, the pace of scientific and technological advances means that IVF practitioners now have access to an increasing array of new and invasive technologies. Examples of these are many but include: extended embryo culture, development of media to include growth factors, developments in genetic screening and use of time lapse technology, and the advent of vitrification of embryos and oocytes. In parallel to this, wider scientific and medical advances mean that we are becoming increasingly aware of the potential impact of assisted reproduction technologies on areas such as: embryonic development, gene expression and genomic imprinting, and the Developmental Origins of Health and Disease (DOHaD). We have recently suggested a paradigm for assessing new technologies in IVF, to include: development in animal models including rodents and large animals, pre-clinical assessment on human embryos donated to research, prospective clinical trials in IVF, and finally, follow up studies of IVF children (Harper et al., Human Reproduction 2012; 27(2): 303-13).

***Susan Bewley***

*Reproductive ageing and clocks: King Midas' touch*

Abstract to follow

***Susan L. Crockin***

*Growing families in a shrinking world: how cross-border surrogacy is challenging actual, legal and ethical boundaries*

International travel for surrogacy and other reproductive treatments is a rapidly growing phenomenon. As "cross-border reproductive care" or "reproductive

tourism" offers options for family building across national borders, it also raises unique legal and ethical issues as laws and cultures inevitably clash. From countries where certain reproductive treatments are expressly outlawed; to others where donors or surrogates are scarce or costs are prohibitively high for many; to those where religious perspectives or public policies exclude some citizens from treatments available to others—patients are finding ways to cross borders, ship their genetic material abroad, and locate surrogate and gamete donors. How these myriad family constellations and efforts to create them are impacted by home and destination countries, how donors, surrogates and intended parents can be legally protected and ethically respected, and how the parent-child relationships and immigration status for these uniquely created global offspring can be legally recognized all challenge existing national and international laws, mores and ethical standards. This presentation will highlight some of the most pressing legal and ethical challenges presented by cross-border surrogacy.

***J.L.H. (Hans) Evers***

*The signal-to-noise ratio of evidence-based ART*

David Sackett provided an excellent definition of Evidence-Based Medicine when he mentioned that it concerns "the integration of *best research evidence* **with** *clinical expertise* **and** *patient values*" (Sackett D et al. in: Evidence-Based Medicine: How to Practice and Teach EBM, 2nd edition. Churchill Livingstone, Edinburgh, 2000). The vaster one's clinical expertise, the better one is able to determine the robustness level required for clinical research to establish the effectiveness of a treatment. The classical example here would be the prevention of gravitational injury by using a parachute during free fall (Smith GC and Pell JP: BMJ. 2003; 327: 1459-61). If the signal-to-noise (S/N) ratio is large, one small and simple study may provide enough evidence for the effectiveness of a treatment (it doesn't take an RCT to prove that parachutes work). A dramatic outcome can be defined by the magnitude of the treatment effect (signal) as compared to the expected prognosis without treatment (noise); some treatments have such dramatic effects that biases can be ruled out without a randomised clinical trial (Glasziou P et al.: BMJ. 2007; 334: 349-51). An example from our field would be the occurrence of a pregnancy after IVF (the signal, or treatment outcome), in a woman without Fallopian tubes (the noise, or natural outcome). In contrast, if the S/N ratio is small, many sizeable clinical trials may have to be performed before enough convincing evidence will be amassed. This unfortunately is the case for most ART treatments. Although a new treatment may seem very attractive and therefore will be easily adopted, its S/N ratio may be low – e.g. in case of IVF in patients with unexplained subfertility. Early adoption of an appealing treatment may thwart the collection of sufficient evidence of its effectiveness.

***Sarah Franklin***

*Conception through a looking glass: the paradox of IVF*

As we enter the fifth decade of human IVF, this technique presents us with a paradox. On the one hand, IVF has become more regular and ordinary, even having become a new norm of social life. On the other hand, as IVF has become coupled to an increasing range of cognate applications such as ICSI, PGD and

gestational surrogacy, as well as human embryonic stem cell derivation, it has arguably become, as Alice might have said, 'curiouser and curiouser'. Five million miracle babies later, in the midst of 'the age of biological control', IVF can be seen as the source of a basic change in how reproductive biology is understood – not only scientifically and medically, but socially, ethically, and economically. This paper argues that while the passage of time may have allowed IVF to become more 'routine', the opposite is also true: with the benefit of hindsight we can also appreciate the radical changes IVF has introduced not only to our understandings of reproduction, but technology, kinship, and genealogy. Learning from this paradox must be part of the legacy of IVF's first half century if its future evolution is to be directed wisely, safely, and conscientiously.

***Alan Trounson***

*A rapidly evolving revolution in stem cell biology and medicine*

The stem cell field accelerated quickly when human embryonic stem cells were reported to be isolated and multiplied as a direct consequence of developments in human preimplantation embryology. The field advanced from a concentration on bone marrow stem cell (hematopoietic stem cells – HSCs) science and medicine to include many other adult and fetal cells to exploring the potential of pluripotent embryonic stem cells (ESCS). With the discovery that adult cells could be dedifferentiated by transcription factor transduction into primitive induced pluripotent stem cells (iPSCs) with properties similar to ESCs. This is a powerful tool to study human disease. Further evolution has taken the field to direct transdifferentiation of cells in vitro and in vivo to cell types of regenerative interest, potentially avoiding immune rejection of transplanted cells. In addition the role of genes in processes of major reconstruction processes in amphibia and fish that have been converted to roles such as tumor suppression in mammals is creating other interesting opportunities in regenerative medicine. At the same time the field is actively exploring translation of stem cell discoveries into clinical medicine. A wide variety of developments may be expected that include; destruction of cancer stem cells, possible cure of HIV-AIDS, reversal of type I diabetes, restoration of vision, repair of motor function in spinal cord injury and heart muscle regeneration.