

Preface

Interest in cell signalling in the early embryo extends over the last 30 years, following extensive research on this topic in somatic cells largely in the context of exogenous growth factors and hormones. Thus, Edirisinghe and Wales (1985), working on preimplantation mouse embryos examined the effect of a variety of agents: glucagon, insulin, adrenaline, cAMP, theophylline and caffeine on glucose metabolism while O'Neill (1985), a pioneer in this field, reported that mouse embryos produced platelet-activating factor (PAF) which stimulated blastocyst rates and cell number. Research then focussed largely on peptide growth factors including their origin—i.e., whether growth factor ligands and/or receptors were produced by the embryo itself or by the female tract (oviduct and uterus), and their cellular effects, which could be autocrine, paracrine or juxtacrine. A number of excellent reviews of these studies were published: e.g., Schultz and Heyner (1993); Kaye (1997); Kane et al. (1997); Hardy and Spanos (2002).

In the last few years interest in embryonic signalling has exploded with (i) the increasing use of ART procedures in animals and humans and the need to improve success rates and safety; (ii) renewed interest in pluripotent cells of the early embryo as a source of embryonic stem cells for therapeutic use in regenerative medicine, drug discovery, toxicology screening, and as a model system for studying early human development; and (iii) the increasing realisation that periconceptional nutrition and environmental stress, experienced both *in vivo*, and *in vitro* during Assisted Reproductive Technology (ART), can act on the embryo to programme long term health (Barker 1989; Watkins et al. 2008; Hart and Norman 2013).

Our aim in this book has been to capture the major approaches being used to understand cell signalling in preimplantation embryos. A key concept throughout the book is that the embryo is *uniquely vulnerable* to stress during the preimplantation phase due to the complete remodelling of the genome and epigenome after fertilisation, coupled with the metabolic demands of embryonic growth, stem cell accumulation and differentiation, implantation and subsequent survival. However, the preimplantation period also affords a unique opportunity for the developing embryo to sense its environment, respond to developmental cues and reset its epigenetic information accordingly. Thus, the book explores our understanding of the sources of stress which act on the embryo, and the way in which the embryo manages

responses to stress in concert with maternal influences. In evolutionary terms there are clear conflicts between the diverse aims of achieving short term embryonic viability to continue a pregnancy, maternal quality control of reproduction such that maternal investment is not wasted on a compromised conceptus, offset against the longer term implications for offspring health. Many of the chapters consider the potential epigenetic mechanisms by which information from the environment may be transmitted to future generations, and the implications of this for human health.

The first chapter, by Eckert, Velasquez and Fleming, provides a most valuable introduction to the morphogenetic processes which occur during preimplantation development during which the one-cell fertilised egg (zygote) develops into the blastocyst, a period which encompasses the onset of polarity and the first major differentiation into an outer trophectoderm and inner cell mass. Control of these processes is considered in terms of intrinsic (intra- and inter-cellular) and extrinsic signals (of maternal origin *in situ* or via the culture environment *in vitro*). The focus is on how the embryo senses the quality of maternal nutrition, especially the amino acid content of the uterine lumen, the role of the metabolic sensor AMPK and on downstream signalling via the mTOR pathway. Overall, the significance of what has now become a lively research area is provided by the concept of developmental plasticity—the notion that the phenotype of the embryo can be modified appropriately in response to maternally derived changes in nutritional environment.

The theme of amino acids as signalling agents between the uterine compartment and the conceptus is developed further in a comprehensive, authoritative chapter by Bazer, Johnson and Wu. Using primarily pig and sheep data they paint a fascinating picture of pregnancy-associated transporters conveying amino acids from the maternal circulation into the uterine ‘histotroph’ and then into the embryo with a pivotal role for mTOR signalling, as in mouse. Aside from highlighting the role of amino acids in ensuring conceptus survival, growth and implantation, this research has identified arginine as a key amino acid whose supplementation in the diet holds promise in minimising early embryo mortality in pig, rodent and human pregnancies and potentially in ameliorating pre-eclampsia in women. As the authors state, this review correctly places amino acids at the forefront of animal (and human) health.

In Chapter 3 the theme of nutrient sensing is extended to glucose. The consumption of glucose by preimplantation embryos has long been known to increase sharply during blastocyst formation—largely, it has been assumed, to provide a substrate for ATP production. However, like many such simple observations and explanations, the reality has turned out to be more complex, but indeed, interesting. As Pantaleon elegantly describes in Chapter 6, glucose is now known to be an integral part of the Hexosamine Sensing Pathway (HSP) which, acting via O-GlcNAcylation, provides a glucose-sensing mechanism which allows the embryo to couple cellular physiological needs with nutrient availability and indeed to integrate responses to other stressors. Perturbed activity of this pathway may provide a link between stress and postnatal outcomes. To this ‘new’ function of glucose in the embryo, one can add its role in the pentose phosphate and polyol pathways, as well as in glycogen synthesis.

Pantaleon's chapter also illustrates the way that the traditional distinction between cell 'metabolism' and 'signalling' is becoming redundant.

Intracellular signalling in response to embryo stress is further explored in Chapter 4, in a fascinating, thought-provoking article by Rappolee and colleagues. The early embryo may be confronted with a variety of stresses: *in vivo* and *in vitro* presented at high and low levels; examples are maternal nutritional and endocrine status and the stress of hypoxia, heat and environmental toxicants. The artificial conditions imposed by ART bring distinct stresses of their own. Rappolee considers the role of stress-related protein kinases and transcription factor-mediated mechanisms, using insights from gene knockouts, and distinguishes 'compensatory' from 'prioritized' responses, focussing particularly on the significant impact of stress on the accumulation and differentiation of the embryonic stem cell population. Increased understanding of these stress mechanisms is essential in devising remedial strategies to improve ART efficacy and safety.

In Chapter 5, O'Neill explores in considerable detail how the preimplantation embryo ensures survival via external, autocrine and paracrine mediators, with a focus on embryo-derived factors such as PAF, the best characterised embryo autotrophin. PAF provides a link between fertilisation and embryonic genome activation. For example via PI3Kinase signalling, PAF induces calcium transients, which in turn activate the transcription factor CREB to generate a diverse, pro-survival transcriptome. If survival signalling is reduced, TRP53 is activated and alters the transcriptome and embryonic fate. O'Neill discusses the role of TRP53 in stem cell accumulation in the embryo, and highlights the clinical risk of embryos with reduced TRP53 activity being positively selected for during ART procedures.

While calcium signalling during fertilisation has been a topic of considerable interest for several decades, knowledge of comparable processes during cleavage, compaction and blastocyst formation is much more limited. Calcium signalling during trophoblast adhesion and invasion has the added complexity of dialogue with the female tract. However, Armant has provided a succinct, first-rate route-map of this journey, in Chapter 6, arguing persuasively that throughout pre- and peri-implantation development, intracellular calcium is a major signalling nexus that co-ordinates intrinsic embryonic developmental programmes in response to extrinsic cues. Thus intracellular Ca is likely to have a pivotal role in co-ordinating the complexity of signalling pathways and responses described in this book.

In Chapter 7, Robertson and colleagues consider the rapidly developing role of cytokines and growth factors in the regulation of embryonic survival and in synchronising development with the maternal environment. In an authoritative, important, account, the authors focus on maternally derived cytokines, in particular Granulocyte macrophage colony stimulating factor (GM-CSF) and make a convincing case that in addition to the above roles, cytokines represent an important mechanism by which the embryo senses its environment, facilitating a plasticity appropriate to prevailing or predicted conditions. Moreover, cytokines may provide a method of maternal quality control of reproductive investment, ensuring that only the viable healthy conceptus survives.

What are the wider practical applications of the work described in this book? These have been carefully considered throughout, and range from understanding the impact of the environment on embryonic development so as to improve ART in animals and humans, management of pregnancy disorders such as intra-uterine growth restriction and pre-eclampsia, and the long term health of offspring. These concepts can also be extrapolated to the naturally conceived population. Potential interventions considered include the use of growth factors in clinical IVF, arginine supplementation in pregnancy, and periconceptual nutritional advice. Ultimately, the aim of the work described here will be to develop interventions which improve animal reproductive biotechnology and health, and human health in the ART and naturally conceived populations.

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Leese, H.J.; Brison, D.R. (Eds.)

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