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Introduction

Epigenetics and periconception environment: an introduction

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Nowadays, epigenetics is a term that is used everywhere, in science and in the popular press. But, what is meant by epigenetics? Why is it always linked with pregnancy and environmental influences? What is meant by reprogramming and prenatal programming? Can a pregnant mother really influence the health of her offspring and what about the role of the father? Is assisted reproductive technology safe for animal production and for infertility treatment in humans? Can we use epigenetics as a tool to improve embryo development and the health of offspring when breeding domestic animals, or can we even use it as a tool to generate healthy cells for medical and veterinary applications? It is true that epigenetic abnormalities have been found to be causative factors in cancer, as well as contributing factors in autoimmune diseases, metabolic pathways and aging. Disruption of the balance of epigenetic networks can lead to inappropriately heightened expression or silencing of genes, resulting in 'epigenetic diseases' (Mutskov *et al.* 2015). In this Research Front with a focus on 'Epigenetics and periconception environment', different aspects of epigenetic mechanisms and how they are influenced by the periconception environment, in the broader sense, are highlighted.

What does epigenetics mean? You recognise two parts in the word: epi- which is Greek for 'on top of', and genetics. Epigenetics refers to reversible marks that are present on top of the DNA, and which influence the expression of genes. Any adult individual has experienced many things that have placed epigenetic marks on his DNA during his lifetime: he has been exposed to pharmaceuticals, environmental changes and other stressors, all of which have left their marks and may contribute to the fact that he will be healthy or not. Sadly, children that have been exposed to suboptimal environments (e.g. malnutrition, environmental toxins or alcohol) before pregnancy (i.e. as gametes), *in utero* and/or during early post partum development have a higher risk of developing conditions such as obesity, insulin resistance and cardiovascular disease in adult life (O'Doherty and McGettigan 2015). This is referred to as Developmental Origins of Health and Disease or the Barker hypothesis (Fleming *et al.* 2015). The attentive reader may now exclaim: 'But you just said these marks are reversible, so adopting a healthy lifestyle must be sufficient to rescue these poor children'! Yes, epigenetic marks are indeed reversible but only to a certain extent. This has been elegantly shown in a study in which pregnant mice were subjected to sleeping disturbances or sleep fragmentation (Mutskov *et al.* 2015). Sleep fragmentation imposes health problems on the offspring of these mice, such as increased bodyweight, altered glucose and lipid homeostasis, and increased visceral adipose tissue. Interestingly, physical activity during early life, but not later, reversed the adverse effects in offspring of mice subjected to gestational sleep fragmentation. The reversibility of this phenotype may reflect epigenetic mechanisms in the offspring induced by the sleep fragmentation of the mother during gestation.

How are epigenetic marks brought about? In general, cells with high plasticity, such as embryonic cells, have more loosely packed euchromatin, which permits active transcription, whereas cells with limited developmental potential have more condensed and tightly packaged heterochromatin, which constrains transcription (Van Soom *et al.* 2014). The level of chromatin condensation and gene expression are controlled by epigenetic marks or mechanisms.

Epigenetic mechanisms play a fundamental role in successful gametogenesis and embryo development. The best studied of these mechanisms is DNA-methylation; the appropriate establishment of DNA methylation patterns in gametes and early embryos is essential for healthy development. These DNA methylation patterns and histone tail modifications may or may not be stably inherited by daughter cells. Most of these epigenetic marks are erased between generations, and this is called reprogramming. The first reprogramming event occurs in the developing gonad. Murine DNA methylation patterns at most sequences are removed during the colonisation of the fetal gonad by post-migratory primordial germ cells (PGCs), at around 9.5–12.5 days post coitum (O'Doherty and McGettigan 2015).

As discussed in the first paper in this Research Front by O'Doherty and McGettigan (2015), it is obvious that during male germline development, paternal DNA methylation marks are erased and established on a global scale through waves of

demethylation and *de novo* methylation. As spermatogenesis progresses the majority of the histones are removed and replaced by protamines enabling a tighter packaging of the DNA and transcriptional shutdown (O'Doherty and McGettigan 2015). Exposure of bulls to heat stress during spermatogenesis can affect protamination of their spermatozoa. The lack of chromatin protamination is the most pertinent consequence of heat stress, together with subtle changes in sperm head shape (Rahman *et al.* 2011). In mice, it has been examined whether stressors imposed upon male mice can be transferred to future generations (so-called 'transgenerational epigenetic inheritance'), which implicates sperm as a mechanism of transmitting heritable non-DNA based information from one generation to the next (Pembrey 2010). This controversial topic is more closely covered by O'Doherty and McGettigan (2015) in this issue.

The maternal germline is also of major interest. Around 3% of human babies in developed countries are born through assisted reproduction, and over 200 000 *in vitro*-produced calves are born each year worldwide. Therefore, it is only logical to investigate whether or not routine techniques such as superovulation or *in vitro* maturation do affect the epigenome of the oocyte. Of special interest here are the imprinted genes, since they are more susceptible to anomalies, with only one allele being active for each imprinted gene. Fortunately, as reviewed by Anckaert and Fair (2015) in this issue, the most recently published data indicate that the oocyte maturation environment within the IVP-system has either no or only marginal effects on the methylation status of imprints H19/IGF2, PEG3 and SNRPN DMRs in oocytes (Heinzmann *et al.* 2011).

When both gametes are formed, fertilisation can occur with a zygote as a result. This entails the occurrence of a second reprogramming event. The mammalian zygote represents a totipotent template harbouring the developmental potential from which an entire organism may be generated and therefore a second wave of genome-wide DNA methylation needs to take place. The classical model suggests that active demethylation in the mammalian murine zygote is considered to result in profound levels of hypomethylation of the embryo creating a near epigenetic 'clean slate' across the genome (except at many imprinted loci) suitable for reprogramming (Salvaing *et al.* 2015). This model implies that during the zygotic cell cycle a marked asymmetric demethylation of the paternal pronucleus relative to the maternal pronucleus is present. This is considered as an active demethylation whereas the demethylation in the maternal pronucleus is deemed a passive demethylation (Gu *et al.* 2011; Iqbal *et al.* 2011; Wossidlo *et al.* 2011). However, this observation has recently been challenged (Salvaing *et al.* 2012; Li and O'Neill 2013), since this classical pattern was different from those observed in other species and because technical changes in the staining procedure can influence the outcome. The review of Salvaing *et al.* (2015) explains how technical aspects of immunolocalisation methods of analysis of global changes in the level of cytosine modifications can affect the interpretation of demethylation events.

The next paper about embryo selection and culture by Gutierrez-Adan *et al.* (2015) does not involve so much epigenetic-related technologies. However, it is known for many years that embryo culture may affect the epigenome of the embryo. It was also thought for many years that fast cleaving embryos were the best to select for transfer. In this review, the authors elegantly show that the fast or slow cleavers are not the best embryos for transfer, but probably the embryos that exhibit an intermediate cleave pattern. Moderately developing *in vitro*-cultured embryos have decreased chromosomal abnormalities, normal *H19* and *Snrpn* imprint maintenance, potentially higher pregnancy rates and may eliminate artificially induced sex selection bias, and are therefore probably the embryos best suited for transfer.

And last but not least is the review on stem cells and phenotypic plasticity by Brevini *et al.* (2015). This review goes beyond the Periconception environment, demonstrating how differentiated somatic cells from an adult can be transdifferentiated into another cell type. A brief exposure to a demethylating agent can push cells to a less committed state, increasing their plasticity. This drug is able to remove epigenetic blocks which are responsible for tissue specification. Once the cells are in this highly permissive state they can be converted into a different phenotype if stimulated with appropriate differentiation media. Functional pancreatic insulin producing cells have been generated using this method, and this opens opportunities for the treatment of diabetes.

In conclusion, this Research Front gives you a hint of what is happening before, during and after conception. It is by far the most important period in the life of an individual. If something goes wrong during this period, there may be long-lasting consequences for the future health of the offspring, such as an increased risk of diabetes and cardiovascular disease. Interestingly, it is also with epigenetic modifiers that such diseases as diabetes may be cured in the near future, with the help of transdifferentiated cells that have been transformed into functional pancreatic islets.

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