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Genomic imprinting – an epigenetic regulation of fetal development and loss

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The interest in genomic imprinting and epigenetics in animal husbandry has to a large extent been driven by the occurrence of a fetal overgrowth syndrome during assisted reproduction techniques (ART) in ruminants. This overgrowth is known as "large offspring syndrome" or LOS for short (reviewed by [1]). It is characterized by a significant increase in birth weight (8% – 50%), increase in gestational length, breathing problems at birth and an increased frequency of perinatal death. The phenomenon has been reported in both cattle and sheep with incidences up to 100%. It has been observed that a high proportion of serum in the *in vitro* culture medium can increase the frequency of LOS in sheep. Thus, factors in the serum and culture-environment per se have been suspected to be involved in the ethiology of LOS, but transfer into an asynchronous uterine environment where the gestational age is unmatched between the embryo and the recipient may also contribute.

Analyses of the molecular mechanisms of LOS in ruminants have suggested that the culture conditions may induce stable changes in the expression of the molecular program inherited from each parent. Such stable changes that do not influence the DNA code itself are often referred to as epigenetic changes. The investigations have also raised the possibility that this artificially induced overgrowth phenomenon parallel some rare birth defect syndromes in humans, the so-called Beckwith-Wiedemann syndrome and Angelman syndrome. These syndromes are caused by mutations in genes that are normally expressed from only one parent, the copy from the other parent being functionally silent. Such genes are known as imprinted genes and constitute a special class of epigenetically modified genes. Thus, for safety investigations of protocol modification in human ART, ruminants could represent a useful animal model. For these reasons, we should have a closer look at what has been learned about genomic imprinting during the last 10 years.

Epigenetics and genomic imprinting – a quick introduction

Mammals have one gene copy from the father (paternal) and one from the mother (maternal). In those cases where they are distinguishable by small neutral differences it has been possible to observe that they are both expressed equally. This is because the regulatory proteins will bind equally to the two gene copies and the number of protein molecules produced from the maternal gene copy is therefore the same as the number of molecules produced from the paternal copy (Fig. 1A). Contrary to this, genes that are subject to genomic imprinting are expressed differently. One of the gene copies is not transcribed, is silent, whereas all protein is produced from the other, active, gene copy (Fig. 1B). Silencing is brought about by a stable mark, an "imprint", which regulates transcription in a region of the chromosome, by interfering with the binding of regulating proteins. For imprinted genes, the difference in expression from the two gene copies is determined by the sex of the parent. Thus, in some imprinted genes it is the paternal copy that is always silenced (the gene is paternally imprinted) and in some genes it is the maternal gene copy (maternally imprinted). For example, in the

insulin growth factor 2 (IGF2) gene, which promote fetal growth, the maternal copy is silenced and IGF2 is thus solely expressed from the paternal gene copy. On the other hand, in the insulin growth factor 2 receptor gene (IGF2R), which inhibit fetal growth, the paternal copy is silenced by imprinting and the IGF2R protein is consequently formed from the maternal copy.

It should be emphasized that it is only a small subset of all the approximately 30.000 genes that are imprinted. In humans some 60 imprinted genes are recorded so far (http://www.geneimprint.com/site/genes-by-species, March 2007). Thus, most genes obey the role mentioned initially, i.e. that both the maternal and the paternal copy are expressed to an equal extent.

There are still many questions to be answered concerning the molecular mechanisms of genomic imprinting. Generally speaking, genomic imprinting is a special case of epigenetic modifications. "Epi" means above, over, outside or beside. Thus, epigenetics literally refers to events that are above or beside genetics, that is, beyond DNA sequence alterations. Three major groups of epigenetic mechanisms are known: Modification of the DNA base cytosin by methylation, modification of the histone proteins of the chromosome by methylation/acetylation and interference of gene transcription by small non-coding RNA. When genomic imprinting is considered, the most usually observed silencing mark seem to be the modification of the cytosin base by methylation. This methylation typically resides in regulating regions positioned in some of the non-coding parts of the imprinted gene, the introns, but there are also examples where it resides in locations far away from the imprinted gene.

The imprint is, as described above, parent-specific. Thus, it has to be laid down in the germ cell at some stage during development. Investigations from mouse have revealed that the imprints received are generally erased in developing germ cells at an early stage of fetal development. Subsequently, identical imprints are formed on all copies of the gene in question, i.e. both the paternal and the maternal gene copy of the germ cells are either imprinted or left non-imprinted depending on the sex of the fetus. This ensures that gametes coming from a male will all carry one chromosome, which has the paternal-specific imprint and that the gamete coming from a female will have the opposite imprinting status.

It seems that the phenomenon of genomic imprinting evolved over 150 million years ago [2]. Thus, genomic imprinting evolved in mammals with the advent of live birth. We can only speculate on its biological significance, but the conflict hypothesis is the most widely favored view at present. This hypothesis states that the evolution of genomic imprinting occurred because of a parental battle/conflict between the sexes to control the maternal expenditure of resources to the offspring [3]. When in accordance with this hypothesis, paternally expressed imprinted genes promote fetal growth while this is suppressed by genes that are maternally expressed. Thus, paternally expressed genes enhance the extraction of nutrients from the mother during pregnancy, whereas the maternal genome seeks to limit it. The basic idea is that the father has a biological, but rather selfish interest in his

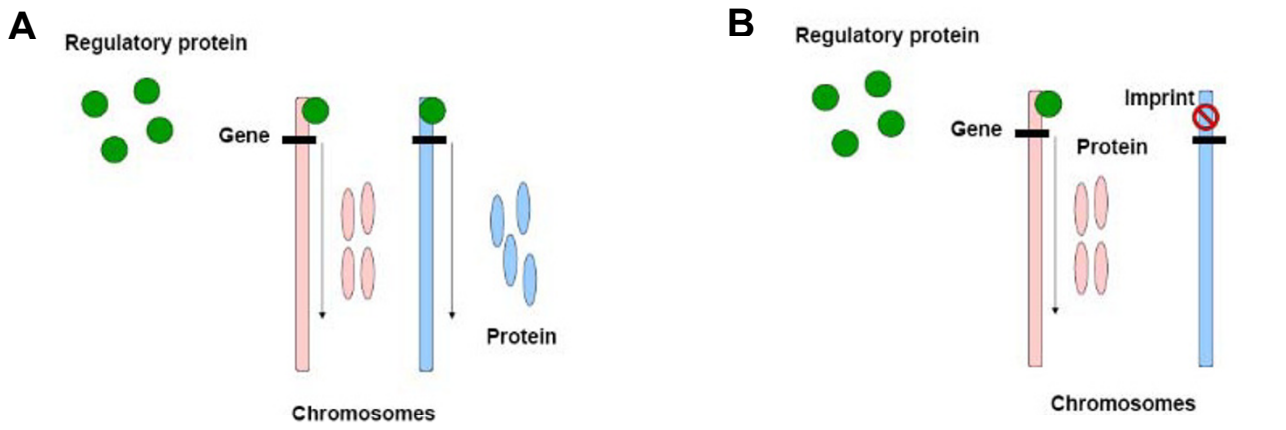

Figure 1
The effect of genomic imprinting on protein production. **(A)** A normal, non-imprinted gene where the speed-limiting regulatory protein bind to equal extent to the two gene copies. **(B)** An imprinted gene, where one of the parents copy is subject to imprinting, which int erferes with the binding of the regulatory protein. Consequently, no protein is produced from the silenced, imprinted allele.

offspring being born (if necessary on the expense of the life of the mother). The mother also has a biological interest in her offspring being born, but also a biological interest in being able to produce more offspring. This genetic battle between the mother and father appears to continue even after birth.

Animal models of genomic imprinting

As described in the introduction, the large offspring syndrome of ruminants may have a mechanism that involves effect of *in vitro* culture on genomic imprinting of the embryo. Young *et al*. [4] reported that a region within the IGF2R gene in sheep, which is imprinted in the mouse, showed differences of cytosin methylations between father and mother. This differential methylation strongly suggests that the IGF2R gene is also imprinted in sheep. The IGF2R inhibits fetal growth by removing IGF2. It is imprinted on the paternal allele and expressed from the maternal allele, thus acting in agreement with the conflict theory. Young *et al*. [4] used *in vivo* fertilized eggs that were cultured in vitro for 5 days in the presence of serum. They recovered fetuses at 125 days after gestation and found 25% of fetuses to suffer from LOS. They reported 30 – 60% reduction of IGF2R mRNA and protein in the fetuses with LOS, and this difference was accompanied by loss of methylation of the maternal copy of the IGF2R gene. Similar alterations of methylation patterns and birth weight in cultured embryos have been reported in mouse [5], thus supporting the evidence from sheep.

These and other findings from animal models have initiated an interest in the possible side effect of human ART on genomic imprinting. Imprinted genes seem primarily to be involved in fetal and in brain development. Thus, imprinting anomalies are often manifested as developmental and neurological disorders when they occur during early development, and possibly lead to increases in embryonic mortality. Specifically, imprinting disorders in humans have been linked to Beckwith-Wiedermann, Angelman and Prader-Willi Syndromes, Alzheimer disease, autism, bipolar disorder, diabetes, male sexual orientation, obesity, and schizophrenia [6]. Reports have shown an increase of some imprinting-associated disorders following ART in humans [7-9]. Although other studies fail to find the same association it has raised some voices of concern in the scientific community (e.g. [10]).

Final remarks

Apart from LOS, there are many other interesting examples of the effect of imprinted genes in farm animals. A phenomenon that has been known for thousands of years via the crossing of horse and donkey is that one obtains two kinds of offspring with differing appearance and differing characters, depending on which species was the father. One gets mules when the mother was a female horse and the father a male donkey. Hinny when the mother was a female donkey and the father a male horse. A plausible explanation of this effect of the species of the father is that there are differences in genomic imprinting in horses. Another example is the link between imprinted genes and muscle development, which has been demonstrated recently in both pigs and sheep. The studies by van Laere *et al*. [11] have shown an influence of the imprinted IGF2 gene on muscle growth and fat deposition. An even more prominent effect is seen of the so-called callipyge mutation on the muscle development of the hind legs of sheep [12]. Callipyge lambs are born normal and the first signs of muscular hypertrophy are first detectable in the loin and hindquarters at 4–6 weeks of age. The mutation is thought to upregulate one or both of the paternally expressed and thus maternally imprinted genes, DLK1 and PEG11. Thus the emerging area of epigenetics holds promises of being of interest to both the farmer, the veterinarian and for the area of animal models for the years to come.

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