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Genetic Factors that Affect Normal Reproduction and Fertility in Domestic Cattle

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The science of genetics concerns the variation of phenotypic attributes encoded in the genetic material and their transmission from one generation to the next. In sexual reproduction systems this is accomplished through the recombination of genes at fertilization, when the new and unique genome or genetic “blueprint” is created, with instructions encoded in the DNA as individual genes. During cell division, DNA is faithfully copied and organized into chromosomes, which appear as identical pairs of autosomes and a pair of sex chromosomes, XX or XY. The number of chromosomes is specific for each species and in domestic cattle (*Bos taurus*) each somatic cell has 60 chromosomes (**2n**), morphologically described as acrocentric, with the exception of the submetacentric sex chromosomes. The gametes have half the genetic material (**n**) and at the time of fertilization they complement each other with one member of each chromosome pair coming from the mother and the other from the father. Variations in the genome of an individual that are not present in the previous generations can occur spontaneously through mutation or chromosomal rearrangement or by DNA technology mediated manipulation.

Fertility is a measure of reproductive success. In males, it can be defined as the ability of a bull to produce semen that will result in a successful pregnancy whereas in females, it can be defined as the ability of a cow to cycle and conceive normally to produce a viable offspring. Such a complex feature is under the influence of numerous genes, working together to produce functional gametes, promote early embryonic and fetal development and finally the delivery of a healthy calf. The heritability, the proportion of variability in a phenotypic trait that is due to genetics relative to the proportion that is due to the environment, is relatively low for fertility, usually with a value of 5% or less ¹. However a number of genetic based variations are known to have direct effects on fertility and reproductive outcome in cattle.

Alterations in chromosome number and structure are the best known of these variations. Numerical chromosomal aberrations can affect the whole chromosome complement (euploidy), which is particularly seen with increasing frequency in embryos as the embryo production system involves more genomic manipulation like in nuclear transfer (NT) ^{15,22}. This type of abnormality is thought to be associated with early embryo loss and return to service. In other instances only one pair of chromosomes (aneuploidy) is affected. Autosomal aneuploidies are found more frequently during the prenatal period, accounting for a high proportion of first trimester pregnancy losses, with trisomies being detected occasionally in live or stillborn

offspring, most of the time associated with gross anatomical defects. Sex chromosome aneuploidy is more frequently seen in the postnatal period ¹⁶.

Structural changes to individual chromosomes result from the “braking” of a part of the chromosome which can be eliminated (deletions), attached to the homologous chromosome (duplication), rotated and attached again (inversion) or be exchanged with another segment from a non-homologous chromosome (translocations). When structural chromosome aberrations do not result in loss or gain of genetic material they are considered “genetically balanced”, and in general are not expressed in the phenotype of the carrier, with the potential to be disseminated in the population if they are not detected ¹⁶.

The most famous example of structural abnormality in cattle is the robertsonian translocation between chromosomes 1 and 29 (1;29). It results in the formation of a large submetacentric autosomal chromosome, with an overall reduction in the number of chromosomes but no loss of genetic material. It was detected for the first time 40 years ago in Sweden ⁹ and has been described worldwide in at least 30 cattle breeds with variable incidence (1 – 65%). The phenotypically normal carriers are associated with low fertility and among its effects are increased female culling rates, increased number of inseminations per conception and lower conception rates ^{4,13}. The defect cannot be detected by the regular reproductive assessment and has the potential of causing important economical losses, especially through the use of affected males in AI programs. After its detection, Sweden implemented a selection program designed to eliminate the use of bulls carrying this abnormality in AI or as replacements resulting in a significant increase in fertility ⁸.

Although humans have been indirectly manipulating the bovine genome for centuries through selection programs based on phenotype and production traits, the essence of this success has remained mostly unknown. Genomics, the branch of genetics that studies organisms in terms of their genomes addressing the molecular basis of phenotype, has seen incredible progress in the last 30 years. Even though currently there are no practical applications in terms of improving reproductive outcome, with the growing amount of detailed information that becomes available everyday, it is predicted that soon we will be in a position to better understand and improve bovine reproductive performance ¹⁸.

Even though many reproductive problems have not yet been associated with particular genes or gene defects, strong genetic associations have been identified. In a Swedish study, AI sires were culled if their daughters exhibited ovarian cysts. Over a 20-year period (1954 – 1974), the national incidence of cysts declined from 10.8% to 3%, implicating genetics as having a major role in the transmission ²³. The advancement of technology in the field of genomics has allowed scientists to explore gene expression profiles to determine which genes are associated in this disease and how they exert their effects ⁷.

The development and application of DNA based technology has made it possible to identify the affected genes in genetic defects of simpler origin. For example, the Deficiency of Uridine Monophosphate Synthase (DUMPS) is a monogenic autosomal recessive disorder that originates from a single nucleotide change or point mutation in the uridine synthase gene in cattle, which results in early embryonic death of homozygous offspring and thus, reduced

fertility of otherwise healthy looking carriers. The mutation has been identified and there are DNA based tests to identify the carriers and avoid their mating²¹.

Genes with positive effects on reproductive potential can also be identified. In a 1993 study two proteins were identified in bovine seminal plasma that were linked to high fertility bulls, and although their function in terms of enhancing fertility in males has not been determined yet, they certainly show potential as molecular markers to be used in future selection of high fertility bulls⁶.

The introduction of the use of Assisted Reproduction Technologies (ART) such as embryo transfer (ET), *in vitro* embryo production (IVP) and NT in cattle has awakened an incredible interest on the gene expression involved with competent gametes and embryo development. These reproductive biotechnologies are having an increasing impact in the field and one good example is that more than 50% of bulls used in the AI industry are derived from embryo transfers.

Successful application of ART requires excellent starting material in the form of competent oocytes and sperm cells. In technologies that involve IVP, the majority of immature bovine oocytes fail to develop to the blastocyst stage following maturation, fertilization and culture *in vitro*. The mRNA transcripts, ribosomes, and proteins accumulated during the growth phase of the bovine oocyte are drawn on to sustain maturation, fertilization, and the initial cell cycle divisions of the embryo up to the 8- to 16-cell stage. Early cleaving zygotes are more likely to develop to the blastocyst stage than their later cleaving counterparts reflecting the intrinsic quality of the oocytes from which they originated. The evidence suggests the intrinsic quality of the oocyte is a key factor in subsequent early embryo development and there is a growing body of evidence showing the effect of oocyte origin and/or *in vitro* maturation conditions on the developmental capacity and gene expression patterns of the oocyte^{5,12,14}.

The effects of post-fertilization environment on embryo gene expression and quality have also been well documented and show that any improvement in the quality of blastocysts produced *in vitro* will come from the modification of post-fertilization culture conditions. It has been shown that after fertilization, the presence of serum in the medium can affect the speed of development and the quality of the resulting blastocysts, resulting for example in a reduced cryotolerance of blastocysts with deviations in the relative abundance of developmentally important gene transcripts. Omission of serum during the post-fertilization culture period can significantly improve the cryotolerance of the blastocysts to a level intermediate between serum-generated blastocysts and those *in vivo* derived. To improve the IVP systems and make these embryos as good as their *in vivo* counterparts it is necessary to identify gene expression patterns associated with competent oocytes and early embryonic development¹⁹.

It has been suggested that the more invasive the embryo production technique, the larger the effect on gene expression. For very intensive genome manipulation applications such as somatic cell NT that rely heavily on IVP techniques, the development of the best culture conditions based on the genetic markers for competency and good development is a must^{3,11,25}.

The progress made in terms of gene sequence information generated by genomic research will allow us to identify gene expression changes due to genome manipulation as in the case of somatic cell NT. For example, The Institute for Genomic Research (TIGR) analyses the public EST sequence data and develop contigs (set of overlapping clones or sequences from which a sequence can be obtained) of sequences for each gene detected within each species¹⁷. Based on the latest releases from the TIGR for cattle there were over 327,000 sequences grouped into 87,257 contigs. With these resources, researchers can observe the expression of literally thousands of genes simultaneously in embryos produced by different reproductive biotechnology applications, measuring the effect of the latter¹⁸.

High throughput gene expression analysis techniques have the power to screen thousands of genes in a single experiment and among them, DNA microarray analysis has features that make it the most attractive method for profiling mRNA expression¹⁰. It has already been used to screen the transcriptome of *in vitro* matured oocytes with very interesting results and this methodology has also been proposed as a tool for studying embryo-maternal communication^{2,10,20,24}. The questions related to gene expression profiles and changes that can be answered with microarray analysis are limitless. This technology will very likely be applied to establish screening genetic profiles associated with high fertility for males and females so that the best males can be incorporated to AI programs and the best females to ET programs or to determine gene expression profiles associated with diseases with polygenic etiology like cystic ovaries.

The genetic factors that affect fertility and reproduction are not limited to those that cause disease. The knowledge generated with the study of gene expression patterns both in germ cells and embryos are leading the way to improve current reproductive biotechnology and will allow us to get the most benefits from their use in practice.

Sommaire

La fertilité, qui est une mesure du succès de la reproduction, est sous l'influence de nombreux gènes qui agissent pour produire des gamètes et des embryons de bonne qualité et est affectée par des anomalies chromosomiques et les maladies génétiques. D'autre part, le développement des technologies du génome et leur utilisation dans l'évaluation des biotechnologies de la reproduction a révélé que la délicate balance génétique conduisant à des gestations réussies peut être altérée par la manipulation du génome auquel ces embryons sont soumis.

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