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Reproductive Technologies and Epigenetics: their Implications for Genomic Selection in Cattle

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ABSTRACT

***Background*:** The development of genomic selection allowing a better selection for multiple traits (both for production and functional traits) induces dramatic changes in the way selection schemes are to be conducted. The associated needs for genomic selection which is to produce a large number of genotyped candidates as quick as possible may/will influence the way reproductive techniques are used to produce them. As the effect of the environment is no more integrated in the evaluation of performances based on genotype, there is also a need to better understand epigenetic effects and their possible implications while implementing genomic selection.

***Review*:** Information brought by reproductive physiology through access to powerful research tools in the fields of genomics, transcriptomics, proteomics and metabolomics will provide new genetic markers and will contribute to improve the precision of phenotypes. The combination of the two types of information is susceptible to increase considerably the efficiency of selection for reproductive traits. As better reproduction may facilitate the way to run selection schemes (more choice among candidates and production of those candidates at a young age), this knowledge can be profitable also to increase the efficiency of multiple trait selection. In this context, and depending on population characteristics, the interest of the reproductive techniques including assisted embryo based reproductive technologies (Multiple Ovulation Embryo Transfer (MOET) and Ovum pick up associated to *in vitro* Fertilization (OPU-IVF)) should be also revisited. The efficiency of systems based on scenarios involving several reproductive techniques taken in combination should be tested. The recent results obtained with embryo typing, which are compatible with the use of the last generation of chips for genotype analysis may lead to very promising applications for the breeding industry. The combined use of several embryo based reproductive technologies will probably be more important in the near future for selection purposes to satisfy the needs of genomic selection by increasing the number of candidates and to preserve at the same time genetic variability. Since several years, genotyping has been used more or less intensively by breeding companies to genotype males and females within nucleus herds As any farmer will get access to the genotype of the females present in their herd, an increased use of embryo based reproductive technologies may result also from the demand of individual farmers who may wish to valorize as well and as quick as possible the genetic potential of their best heifers following genotyping. In the near future, a better knowledge on epigenetics will allow to estimate the interactions between genotype and environment and their impact on performances of present or future generations. This represents a critical information when evaluating performances and when selecting future sires on genomic based information especially with the objective of implementing sustainable breeding schemes.

***Conclusion*:** The manuscript describes the new context and corresponding needs for genomic selection and how reproductive technologies and additional knowledge on epigenetics can be used to meet those needs.

Keywords: genomic selection, gene expression, transcriptomics, epigenetics, phenotype, reproduction, embryo, biotechnology.

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I. INTRODUCTION

During recent decades, advancement in our knowledge of reproductive physiology and regarding improvements in embryo-based reproductive biotechnologies have facilitated the development of a rather complete "tool box" including reproductive techniques used either for commercial purposes and/or in the frame work of breeding schemes. These techniques currently have varying degrees of efficiency [52] and for most of them continuous improvements may be expected in the future. Used alone or in combination, their development is influenced in many different ways including ethics and general acceptance, consumer demand for specific products, regulatory changes and also changes related to the evolution of breeding strategies.

The recent development of genomic selection has lead to dramatic changes in the way genetic selection schemes are to be conducted [7,30]. Due to the present and expected evolution in the organisation of selection strategies and associated requirements, the value of the various reproductive techniques used today for commercial purposes and in genetic schemes will be revisited. The need to better understand epigenetic effects and their possible implications while implementing genomic selection will also be discussed.

II. THE NEW CONTEXT AND NEEDS FOR GENOMIC-BASED SELECTION SCHEMES

In genetic selection the expression for a given trait is the phenotype that integrates the effect of genes and the effect of environmental factors. In the past the effect of the genetic component was evaluated from genealogy and by measuring performances/phenotyping of candidates or of their progeny. Today, in association with information issued from genealogy, genomic selection, while linking from previous experience, the presence of genes and/or polymorphism of those genes to performances, allows to predict the genetic value of a candidate which is revealed by the presence of pertinent markers indicative of its genotype.

Marker Assisted Selection (MAS) has first been developed and was based initially on a limited number of micro satellite analyses for a few Quantitative Trait Loci (QTL). Selection was performed by combining this first generation of genomic information with conventional indexes arising from quantitative genetics. Further developments of genomic selection were made when it was shown [48] that it was possible to predict the total genetic value of animals or plants by using genome-wide dense marker maps. The progress of the knowledge of the bovine genome and of DNA analyses has made dense marker maps available in this species and the position of markers in relation to genes of interest has been refined. This allows animal breeding companies to use today sets of thousands of genetic markers to select animals [8,14,19,25,50,58].

The development of genomic techniques will probably make available the use of the complete genome information for selection purposes in a few years [15]. Different types of chips based on the use of Single Nucleotide Polymorphism (SNP i.e a single base difference on the DNA between individuals or groups of individuals) can be used to achieve different objectives. The bovine 50K SNP chip is today the standard tool for breeding industries in dairy cattle and all the traits previously selected through quantitative genetics can now be evaluated from genomic information [20].

In parallel with those technological changes the value of the genomic information is reinforced by including more and more animals in the evaluation and selection process [8,14]. Consequently, more reliable estimates can be obtained for the desired traits while genetic variability is better preserved. Candidates will have to be produced from parents of different pedigree's (maximum of families within a breed) and at the same time breeding should be organised in a way to maximize the variability of the next generation [9]. Due to its costs and to the fact that the genetic value of a given future sire is known with enough precision from genomic analyses, the need for progeny

testing will be considerably reduced or even removed [7].

For some traits, such as those related to fertility, the precision associated with genomic indexes is or will be much better than with classical selection [4,20]. Efforts are also made to build a common reference basis in different populations to optimize the evaluation process and evaluate the changes induced by genomic selection [11]. Research is made also on computational methodologies to define the best way to analyse and use the huge quantity of information arising from genomic analyses of a very large number of animals [14].

For all traits of interest, these changes highlight the importance of the phenotypic information that must be unified from large number of animals in the reference base and which becomes one of the main bottlenecks in the process.

III. HOW REPRODUCTIVE PHYSIOLOGY AND TECHNOLOGIES CAN HELP TO MEET THE NEEDS OF GENOMIC SELECTION AND COMMERCIAL PRODUCTION?

3.1 Reproductive physiology

In an attempt to improve numerous traits by genomic selection knowledge of the relationships between genome information and phenotypic criteria is of crucial importance. Initially, microarrays were used to characterise the relationships between genotype and phenotype [1,2,16,36]. More recently, high throughput technologies for DNA sequencing and RNA analysis are now currently used to study relationships between genotype and phenotype and gene expression.

With such objectives, phenotyping (animal models, precise criteria and methods) becomes the main bottleneck to achieve this goal. As a consequence, research is needed and performed to phenotype new critical traits and/or to improve the precision of the phenotypes for existing traits such as fertility and reproductive traits [10,27,31,32]. For this purpose, proteomics, lipidomics and metabolomics may be particularly appropriate to find new markers for fertility [10].

3.2 Use of embryo based biotechnologies

One of the most important features of the new selection procedures will be to considerably increase the number of candidates submitted to genomic selection to maximize the chances of getting interesting individuals that will be positively evaluated for a large number of traits. As mentioned before, this will allow an increase in the selection pressure for those traits. Also, it will be possible to use bulls for AI at a younger age, thereby lowering the generation interval. Finally, the use of groups of bulls with a favourable genomic index will improve the precision of indexes when compared to the use of a very limited number of older sires as was the case in the past. This may be also favourable to genetic variability if adequate and wise breeding schemes are implemented; otherwise shortening the generation interval may also lead to an increased inbreeding rate.

The way to produce these large numbers of animals becomes critical. In this context, AI alone may be inadequate to generate sufficient animals in a given period of time and the efficiency of MOET and OPU-IVP looks more and more critical to produce these large numbers of animals to be genotyped.

With these "intensive" embryo-based reproductive techniques it is relatively easy to increase the number of candidates by increasing the number of flushes in MOET schemes. When compared to MOET, the number of embryos produced in a given period of time can even be multiplied by 2 or 3 [46,52] by the use of repeated OPU-IVF sessions. This method presents also advantages to preserve genetic variability. A lot of research has been done to improve *in vitro* culture systems. This allows most teams working with IVF to obtain overall development rates up to the blastocyst stage between 30 and 40%.

The effect of a previous superovulation on fertilisation and subsequent embryonic development is still controversial [52,56]. It has been shown very clearly from most studies that there is a significant decrease in embryo production when oocytes are matured *in vitro* in standard medium compared to *in vivo* conditions [12,26,28,56]. This emphasizes the roles of the final steps of oocyte growth and maturation in subsequent embryo development which have also been illustrated by epidemiological studies showing relationships between some factors influencing these steps and embryonic mortality [32].

There is probably a lot of progress that can be achieved *in vivo* and *in vitro* embryo production by optimizing the conditions under which the oocytes are growing within follicles in donor females. Handling at the

time of collection and thereafter as well as *in vitro* maturation are also critical steps to be optimized since dramatic metabolic changes occur very quickly after oocyte recovery [21].

Despite the above mentioned limitations and potential margins for progress, the work that has been done in the past 15 years to improve oocyte collection and *in vitro* embryo production systems has made those systems used by the most advanced breeding companies to produce more embryos in their genetic schemes [46,59].

However, i) to mis-manage the use of these techniques may lead to increase inbreeding significantly especially if bull dams are overexploited (Colleau 2010, personal communication) and ii) due to the new requirements in relation to the implementation of genomic selection (increased number of candidates), additional strong limitations exist for giving birth to a very large number of calves that would be genotyped after birth. Effectively, one of the main bottlenecks experienced by breeding organisations working in Europe with dairy cattle is the limited availability of female recipients. This is reinforced by the fact that, due to lower pregnancy rates when using cows instead of heifers as recipients, the efficiency of embryo transfer is much lower if the heifers are used mainly as donors and not as recipients [52].

In addition to this, high costs will be induced by the transfer of a very large number of embryos into recipients that must be maintained pregnant until birth of progeny and the economic potential of the non selected calves will be low. When producing these candidate animals on farm, the amount of field work in relation to embryo transfer and *in vitro* production will be even greater than today and will generate high logistical costs.

Finally, this process may increase the contractual cost with individual farmers especially due to the potential existence of very interesting candidates identified by genomics. For these reasons, genotyping the embryos and selecting them before transfer appears to be an attractive scenario to maximize the chances to finding interesting individuals for multiple traits while transferring a "reasonable" number of embryos.

3.3 Embryo Typing

The interest of embryo typing for breeding companies was discussed even before the emergence of the new techniques for genomic selection that includes today thousands of markers [46]. Doing typing and selection early in life was expected to be a solution to shorten the generation interval and to limit the costs of producing the high number of calves and of progeny testing to achieve multi character selection. Today the potential advantages of combining intensive embryo production and genotyping are even higher.

Results reported initially in the literature for ruminants were based on the typing for a limited number of markers [23,29,39,40,51]. Field studies with *in vivo* produced, biopsied embryos either fresh or frozen have shown that pregnancy rates following the transfer on farm were compatible with field use [22,37,53] (Table 1).

Initially typing was envisaged from a large number of cells issued from reconstituted embryos following cloning of blastomeres [39] (Figure 1). As preliminary studies from limited numbers of biopsies and typing have shown that the use of pre amplified DNA is possible [22] and compatible with the typing from high density markers chips [41] it may be useful to perform economic and genetic simulations to precisely evaluate the costs and advantages for the genetic schemes of such procedures based on embryo typing.

3.4 Other reproductive techniques

To a certain extent, sperm sexing can help to limit the number of embryos to be produced for this purpose and may be used in combination with *in vitro* fertilisation - *in vitro* production (IVF-IVP) procedures. Use of semen sexing in association with IVF-IVP may also avoid some of the present limitations of the use of semen sexing in selection schemes in relation to the high number of sperm that must be discarded and the large individual variation associated with the sexing process by flow cytometry [54].

Finally, considering the need to maximize genetic variability and due to strong limitations in reproductive efficiency, cloning is unlikely, at least at present, to represent a useful tool in the frame work of selection schemes. However, besides selection schemes driven by breeding associations / companies, individual farmers that may get access to genomic selection, may be interested in the duplication of their best animals with the help of cloning for commercial purposes in countries allowing the use of this process.

IV. IMPACT OF GENOMIC SELECTION ON REPRODUCTIVE TECHNIQUES

4.1 Genetic Schemes

Artificial Insemination (AI), Multiple Ovulation and Embryo Transfer (MOET) and Ovum Pick Up associated with *in vitro* Embryo Production (OPU–IVP) have been used more or less intensively to

Table 1. Pregnancy rates (day 90 post transfer) following transfer of biopsied embryos either fresh or frozen. Results from (*) and 1 were obtained after embryo sexing. Results from series (2) were obtained after biopsy and typing ffor 45 microsatellites (detection rate 98%), from [22].

Type	Frozen		Fresh	
Location	No	% Preg. (D90)	No	% Preg. (D90)
Total farm (*)	61/116	52.3	109/171	63.7
Total station (1)	46/83	55.4		
Total station	28/54	52		
Total	135/253	53	109/171	63.7

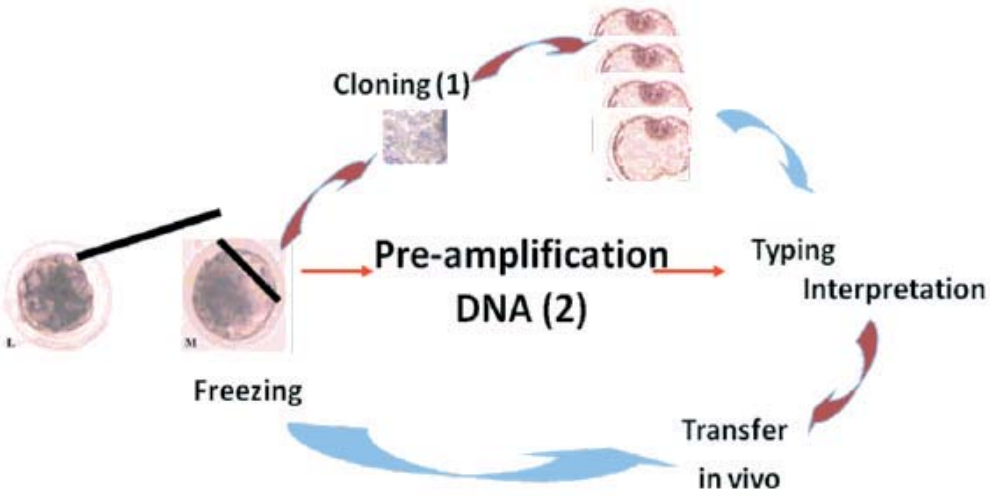

Figure 1. Typing the embryo either from cloning blastomeres issued from biopsies (1) or following preamplification of DNA issued from few cells [22, 41].

generate the future sires following selection through highly effective and very costly progeny testing programmes. The changes in breeding strategies and use of reproductive techniques associated with the needs of genomic selection will make the use and efficiency of embryo transfer, OPU and IVF very critical and these techniques will be probably more used than in the past to increase the number of candidates [24,47].

On top of this, the potential value of the genotyped animals will probably lead breeding associations/ companies to adopt strategies allowing them to control the production of genome-selected animals through use of embryo-based reproductive techniques (MOET and IVP) in nucleus herds to give birth to previously (pre) selected animals within a given structure / company and not on farm.

4.2 Commercial activity

As soon as genotyping will be extended, farmers will have access to the corresponding information in females. This will probably induce a strong rise in the demand for ET and even OPU and IVF from farmers wishing to optimize the value of their best females within their herds and/or for commercial purposes.

V. EPIGENETICS: BASICS AND PERSPECTIVES

Epigenetic changes can be defined as stable alterations of DNA associated molecules but that do not involve changes of the DNA sequence itself. As a consequence to those changes, the expression of genes can be altered due to modifications of active regulatory sequences inducing alterations of the cellular phenotype which can lead in turn to

modifications of animal phenotype. Epigenetic alterations involve changes in DNA methylation (including global hypomethylation and more locus specific hypermethylation) and methylation or acetylation of histones [34,49] (Figure 2).

Epigenetic alterations happen in all cells in the body. If they occur in egg cells or sperm they can be passed on to the next generation. The epigenome is especially vulnerable during embryogenesis, fetal and neonatal life and at puberty [3,18,33]. Effectively, the epigenome undergoes extensive reprogramming when the gametes (eggs and sperms) are formed at fertilization (during the final stages of meiosis and around fertilization due to chromosomal decondensation and intense remodelling) and during the preimplantation period. These epigenetic changes are necessary for normal embryonic development and survival [33]. The modification of epigenetic marks that occurs during pre implantation development correlates with the activation of the embryonic genome [38,45].

After fertilization, different patterns of methylation exist for some epigenetic markers followed by more or less early demethylation which allows transcriptional activity of the embryonic genome [6]. Later, at the morula stage, DNA

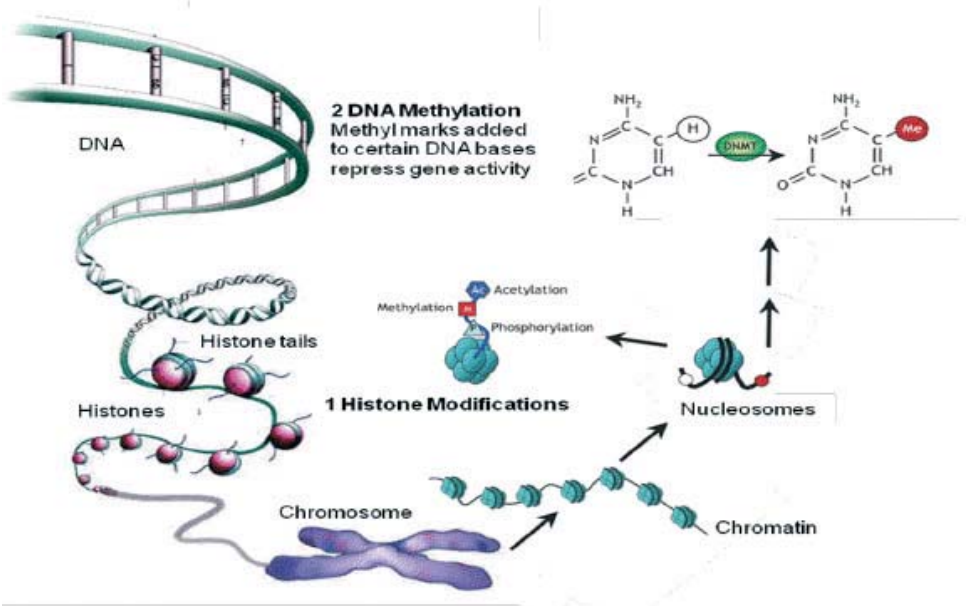

Figure 2. The 2 major components of the epigenetic code [34, 49]. 1) Methylation of histones influences chromatin structure and nucleosomal remodelling and 2) Methylation of DNA bases. Changes in methylation are associated with chromosomal instabilities due to repression of repair DNA genes in the case of hypermethylation whereas hypomethylation leads generally to up regulation and over expression of some genes. Histone modifications leads to either a more condensed state of chromatin structure associated with transcriptional repression (methylation) or to a more relaxed state associated with transcriptional activation (acetylation). These different modifications are closely interconnected as the methylation status influences histone status and some regulators of nucleosome remodelling controls also methylation and histone modifications.

methylation at specific loci influences differential gene expression patterns of the cells at the periphery of the embryo [57], whereas cells at the center of the embryo do not receive the same environmental influence and instead conserve their totipotency. Reproductive technologies, such as *in vitro* fertilization, cloning by nuclear transfer in domestic animals and assisted reproduction technologies (ARTs) in humans, are used during stages of fertilization and early embryo development, when a potential window of vulnerability exists. These techniques have been used as experimental models for the role of epigenetic effects on embryonic development and a large number of studies demonstrate the impact of ART on gene expression in the mouse [13,17,35,42,43,55]. In the mouse and cow, epigenetic modifications induced by ART were associated with impaired early embryonic survival but also with deleterious effects on further post-implantation, fetal, placental and postnatal

development [5,17,44]. In those species, as well as in humans, some of the alterations may be due to changes related to the epigenetic regulation of endometrial function [49].

In brief, epigenetic effects can influence i) reproductive efficiency (through alterations in the viability of embryos, foetus and new born and control of endometrial gene expression which may alter implantation) ii) health (especially occurrence of cancer through regulation of proto oncogenes and suppression of tumor suppressor genes) and iii) phenotypic performance for a variety of functions/traits. Some epigenetic modifications can be even transmitted to next generations leading to remanent alterations of the phenotype within families.

Numerous factors can induce epigenetic effects and for instance evidence has been shown for the role of i) nutrition (either overnutrition increasing the rates of diabetes and obesity or undernutrition) as nutritional challenges to the established germ cells can determine the chromatin structure leading to metabolic responses throughout life in an individual, ii) endocrine disruptors (which can induce durable changes in receptor sensitivity to steroids (androgens and oestrogens) that may be involved in cancer occurrence) and iii) for various pollutants in inducing such effects.

This information shows that epigenetic regulation of gene function is a key factor to understand the interactions between environment and genome function. This has clear implications in selection especially today when using genomics based on DNA sequence characteristics to predict future performance instead of observed phenotypic performances of offspring through progeny testing which were integrating potential epigenetic effects in the evaluation.

Considering the information given above, when using Assisted Reproductive Technologies, a better knowledge of epigenetic effects will help also to better define culture conditions for oocytes and embryos that will not impair subsequent embryo, foetus, new born development and health of offsprings. More generally this knowledge may help to define preventive measures which may be favourable to fertility and health for a variety of species including man.

VI. CONCLUSIONS

In the new context of genomic selection, there is still a lot of work for the reproductive physiologist to study gene expression and identify markers and networks of genes associated with fertility. As far as selection for fertility is concerned, more precise phenotyping is needed for particular reproductive events and more especially for precocity of reproductive traits that has not been well characterized so far.

More generally, for all production traits and functional traits, in the present context showing very impressive improvements induced by the intensive use of MAS, it is likely that the use of a set of intensive reproductive techniques together with embryo typing will bring very significant advantages to breeding organisations capable of monitoring all those techniques with efficiency and to implement them in selection schemes. However, strategies must be developed to use all these techniques in such a way that they contribute to maintain genetic variability.

There will probably also be some changes in relation to commercial activity due to valuable genomic information becoming available in females that may lead individual farmers/companies to make a larger use of semen sexing and embryo related technologies.

Knowledge about epigenetic effects on various functions will help in many ways to better integrate environmental effects in the evaluation system when using genomic selection in farm animal species and more generally to define preventive measures to optimize reproductive efficiency and health. To achieve this last objective, a comparative approach involving many species as different models to investigate such epigenetic effects within different tissues (which are more or less accessible depending on species), will be probably profitable.

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