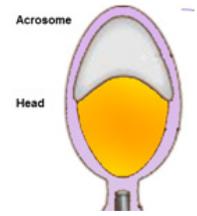


# Dairy cattle reproduction is a tightly regulated genetic process: Highlights on genes, pathways, and biological processes



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## Implications

- Herd fertility is a key factor for the sustainability of cattle farming. However, declining fertility has become one of the main causes of culling and replacement of cows in developed countries.
- Omics technologies have been successfully developed in cattle, refining our knowledge of molecular mechanisms governing cattle reproduction. However, most studies have failed to establish clear links between the genome and final phenotypes.
- Further studies will be needed to integrate all Omics data and develop a systems biology modeling approach to tackle this complex biological function.

**Key words:** environmental factors, genetic factors, Omics, reproductive performance

## Introduction

Herd fertility is a key factor for the sustainability of cattle farming. However, since the 1980s, fertility in dairy cattle has steadily decreased and has become one of the main causes of culling and cow replacement in developed countries (Lucy, 2001; Barbat et al., 2010). Many studies have been performed in the last decades to investigate the genetic bases of this decline, considering the reproduction process as a whole or focusing on some particular stages of reproduction. A better knowledge of molecular mechanisms was expected to help in identifying key genes and explaining antagonistic interactions between fertility and other traits exemplified by the negative genetic correlation between milk yield and fertility in dairy cattle. For instance, spermatozoa or oocyte quality and their interactions with the maternal environment, as well

as embryo and fetus developmental capabilities, have been assessed using the so-called “Omics” in various dairy cattle breeds. Omics refers to the high-throughput technologies such as microarrays or new sequencing technologies (NGS) used to explore the genome structure (genomics) and function (transcriptomics, proteomics, and metabolomics). Epigenetics studies also provide some insight into the role of heritable changes in gene expression due to cytosine methylation as well as long and small non-coding RNAs. Both gene or quantitative trait loci (QTL) mapping programs and functional genomics studies have been performed to search for key genes associated with reproduction and fertility, and many results have been obtained. Unfortunately, no clear picture has emerged. However, incorporating reproductive traits in genomic selection programs has succeeded in reversing the declining trend in reproductive success in many countries such as the USA, where the genetic merit for dairy cattle fertility has now reached 1985 levels. Consequently, reproductive success in dairy cattle is now seen as the result of complex and dynamic interactions between the genome and environmental factors.

The aim of this review is to provide an overview of genes and functions associated with male and female reproduction, highlighting whenever possible candidate genes and variants responsible for genetic variations in fertility. Due to the huge amount of data and literature, we focused mainly on studies not described in previous reviews. As a result, this review is not meant to be exhaustive but will demonstrate the value of genetics and genomics to understand reproduction processes and finely decipher their molecular bases in cattle.

## Studying Male and Female Reproductive Performance as a Whole

A few parameters are computed to evaluate male fertility, including non-return rate (NRR), estimated relative conception rate (ERCR), and male reproductive ability (MRA), which is adjusted for environmental and female genetic effects. Testicular development and spermatogenesis have also been investigated as predictors of male reproductive performance (for review, see Burns et al., 2011). Female performances are evaluated through numerous parameters from conception to weaning: age at puberty and at

first conception, ovulation rates, fertilization failure, number of inseminations, embryo death, fetal losses, gestation length, duration of post-partum anoestrus, fertility, and interval between first and last service (review in Fortes et al., 2013).

### Omic of male fertility

Only a few genetic studies (Genome-Wide Association Studies or candidate genes) have been dedicated to male fertility in cattle (review in Fortes et al., 2013). Results are summarized in Table 1. Highlights included FGF2 and STAT5A gene polymorphisms associated with ERCR and milk production in Holstein cattle (Khatib et al., 2010) and a missense mutation in the PROP1 gene, associated with sire conception rate (SCR) and milk production in the Holstein breed, thus providing molecular evidence for the antagonistic relationship between milk production and fertility (Lan et al., 2013). In addition, idiopathic subfertility in Fleckvieh bulls was linked to a causative loss-of-function mutation in the TMEM95 gene (Pausch et al., 2014). A multi-species, genome-wide comparison gave a comprehensive overview of male fertility-associated loci, providing a list of 835 functional candidate genes from seven species, giving the opportunity to identify novel biomarkers to improve fertility in domestic animals (Ogorevc et al., 2011).

The presence of mRNA transcripts in sperm is now well acknowledged, but their putative roles are unknown. Several hypotheses have been suggested, considering that they are roughly similar to those found in testis (remnants mRNA) and may thus reflect the accurate development of spermatogenesis. An alternative hypothesis suggests they may play a role during the first steps of fertilization. One transcriptome analysis identified 415 differentially expressed transcripts from high- and low-fertility Holstein bulls (Feugang et al., 2010). Semen from high-fertility bulls contained higher concentrations of mRNA coding for transporters and translational factors while genes involved in transcription, protein binding, and cell cycle were upregulated in low-fertility bulls. Thus, a great proportion of differentially expressed genes may have crucial roles at the moment of fertilization, supporting the alternative hypothesis.

Proteomics was also used to identify putative biomarkers associated with fertility (review in Rahman et al., 2013). In particular, 125 differing proteins have been identified between high- and low-fertility spermatozoa (Peddinti et al., 2008), with higher expression of proteins involved in energy metabolism, epidermal growth factor (EGF), platelet-derived growth factor (PDGF) signaling, spermatogenesis, and cell motility in high-fertility spermatozoa. A cross-breed comparative study also identified 44 differentially expressed proteins between high- and low-fertility cattle, including a number of motility- and energy-related proteins (Ashrafzadeh et al., 2013). Likewise, several proteins regulating metabolism, oxidative stress,

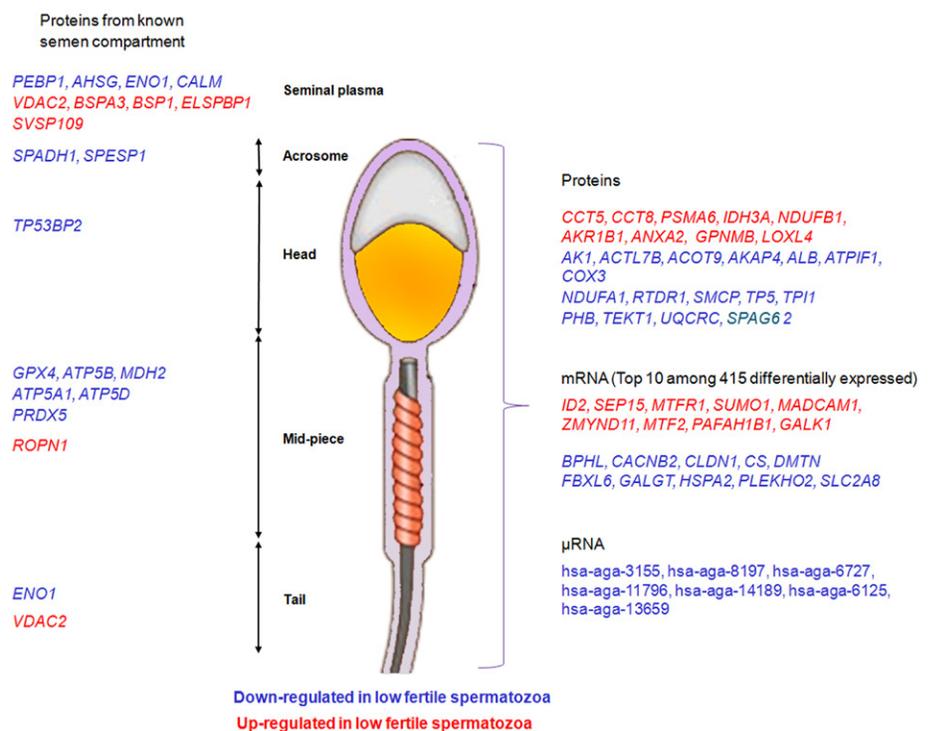
**Table 1. Chromosomal regions (QTL, quantitative trait loci) associated with male fertility, which have been identified in different dairy cattle breeds from GWAS-based studies.**

Trait	Breed	Chromosomes	References
RSCR	Holstein	1, 4, 9, 21 and 27	(Michot, et al., unpublished)
RSCR	Normande	1, 2, 5, 8, 10, 15, 17, 19, 22, 23 and 25	(Michot, et al., unpublished)
RSCR	Montbéliarde	2, 3, 4, 7, 9, 13, 19 and 29	(Michot, et al., unpublished)
SCR	Holstein	2, 5, 18, 25 and 29	(Penagaricano et al., 2012)
SCR	Holstein	7	(Lan et al., 2013)
Estimated RSCR	Holstein	1, 17 and 19	(Khatib et al., 2010)
Male reproductive ability	Fleckvieh	19	(Pausch et al., 2014)

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posttranslational modification, and motility of spermatozoa were found to be modulated in low-fertility bulls (Park et al., 2012; Soggiu et al., 2013), including voltage-dependent anion channel 2 (VDAC2). Interestingly, chemical inhibition of VDAC2 and VDAC3 proteins was recently shown to significantly alter spermatozoa functions as well as fertilization and embryo development, suggesting that they are essential for successful reproduction (Kwon et al., 2013). In addition, repertoires of proteins from epididymal fluid (Moura et al., 2010) and seminal fluid (Druart et al., 2013) were also recently established, paving the way for the study of their role in sperm maturation and fertility.

A novel research area has emerged in the last years, focused on epigenetic mechanisms involved in sperm production and their impact on the differentiating embryo. Histone-to-protamine transition, histone modification, DNA methylation, and noncoding RNAs have important, but so far underestimated, roles in the production of fertile sperm. A repertoire



**Figure 1.** Previously published studies were used as a reference to summarize the list of protein downregulated (in blue) and upregulated (in red) in low-fertility spermatozoa. Proteins are described in Roncoletta et al., 2006; Peddinti et al., 2008; D'Amours et al., 2010; Park et al., 2012; and Soggiu et al., 2013. Transcripts were identified by Feugang et al., 2010 and miRNA by Govindaraju et al., 2012.

**Table 2. Some additional chromosomal regions associated with female fertility. Either locations (Mb) or genes names are reported. When several QTLs are located on a same chromosome, their locations are separated by a '+' sign.**

Trait	Breed	Chromosome number (location / Gene)	References
Female fertility	Montbéliarde	1 (68Mb), 10 (45Mb), 18 (55Mb), 19 (64Mb)	(Fritz et al., 2008)
	Normande	2 (18Mb), 4 (69Mb), 7 (92Mb), 20 (70Mb)	(Fritz et al., 2008)
	Holstein	1 (8Mb), 2 (18Mb), 14 (42Mb), 18 (68Mb)	(Fritz et al., 2008)
	Holstein	19 (ACACA), 20 (PRLR)	(Fontanesi et al., 2014)
	Nordic Holstein, Jersey and Red	1 (89+95+106 Mb); 3 (36+59+96 Mb); 4 (22+47 Mb); 7 (39+41+93 Mb); 8 (59+89+102 Mb); 9 (54+96+101 Mb); 10 (34 Mb); 11 (19+82 Mb); 12 (90 Mb); 13 (60 Mb); 20 (66 Mb); 23 (49 Mb); 24 (21 Mb)	(Hoglund et al., 2014)
Calving rate	Holstein	1 (6+8+141; 3 (26+86); 4 (66); 6 (92+96); 18 (49); 26 (41)	(Lefebvre et al., 2011)
NRR56 cows	Nordic Holstein, Jersey and Red	1 (84+125+135Mb); 3 (44+90Mb); 4 (67+73Mb); 8 (8Mb); 9 (61+103Mb); 11 (2+58+95Mb); 16 (59Mb); 17 (12Mb); 22 (60Mb); 23 (46+51Mb)	(Hoglund et al., 2014)
NRR56 heifers	Nordic Holstein, Jersey and Red	1 (87Mb); 2 (46Mb); 3 (10Mb); 5 (15 + 37Mb); 8 (21Mb); 11 (30Mb); 13 (56+71Mb); 14 (16Mb)	(Hoglund et al., 2014)
NRR90 NRR282	Holstein	3 (26Mb)	(Guillaume et al., 2007)
NRR282	Holstein, Normande, Montbéliarde	7 (107Mb)	(Guillaume et al., 2007)

of bovine spermatozoan microRNA (**miRNA**) has been established and seven miRNA showed differential expression in sperm from one high- vs. one low-fertility bull (Govindaraju et al., 2012).

Altogether, functional genomics studies have provided large lists of candidate genes, suggesting potential fertility biomarkers, but no common biomarkers could be identified from transcriptomics and proteomics published data. Proteins involved in energy metabolism seemed to be associated with fertility, in good agreement with the crucial role of glycolysis and ATP production to support sperm function. In contrast, transcripts showing the highest changes in expression appeared to be probably associated with fertilization and early embryo development. However, most of these results were obtained by comparing a limited number of “high” versus “low” fertility bulls (Figure 1). Further studies on individual proteins and a search for DNA polymorphisms will be required to assess their association with sperm fertility in large pedigrees.

### Omics of female fertility

Female fertility was mainly studied from a genetic point of view. Quantitative trait loci associated with different aspects of female fertility and successful gestation have been evidenced (review in Fortes et al., 2013), and most of the results have been gathered in the CattleQTLdb database (<http://www.animalgenome.org/cgi-bin/QTLdb/BT/index>). Unfortunately, less than 20% of QTL regions have been found in common between populations. In the last years, fine-mapping programs refined some of the initially described QTL regions, which were subsequently confirmed in independent cattle populations. A GWAS study of Danish and Swedish Holstein cattle reported short QTL regions on several chromosomes, facilitating the search for candidate genes and possible causative polymorphisms (Sahana et al., 2010). A recent association study identified a total of 4,474 genome-wide significant SNP associated with eight heifer and cow fertility traits in the Nordic Holstein population, 836 and 686 of them being also confirmed in Nordic Red and Jersey cattle breeds,

respectively (Hoglund et al., 2014). Table 2 summarizes some additional results, which were not reported in previous reviews.

## Focusing on Gamete Quality

Spermatozoa and oocyte intrinsic quality are crucial for fertilization to succeed. Interactions with the parental environment also play a significant role for gametes to acquire their developmental competences (capacitation and maturation).

### Semen quality

The capacity of sperm to reach the site of fertilization and fertilize the egg is a key factor for male fertility. Several laboratory assays are used to assess semen quality and production, including compensable traits (i.e., concentration, motility, and morphology) and non-compensable traits that lead to subfertility (i.e., nuclear vacuoles, defective chromatin structure). Genetic determinants of such traits have been analyzed and QTL associated with semen quality, sperm volume, and survival after thawing have been identified (Druet et al., 2009; review in Fortes et al., 2013). We also recently reanalyzed the initial dataset of Druet et al. using SNP (Michot et al., unpublished), identifying 6 to 10 QTL for each semen quality trait, including 3 QTL already reported (Table 3).

Furthermore, a study of 15 cattle breeds revealed significant copy number variations (CNV) for the PRAMEY (preferentially expressed antigen in melanoma, Y-linked) gene, which correlated negatively with scrotal circumference, percentage of normal spermatozoa, and NRR, suggesting that they may serve as a valuable early marker for sire fertility selection (Yue et al., 2013). However, studies are still required to precisely evaluate the link between sperm fertility and these quality parameters, especially at the molecular level.

**Table 3. QTL detected for several semen quality traits.**

Trait	Breed	Chromosomes	References
Non-compensatory fertility	Holstein	3 to 6, 8, 10, 12 to 15, 17, 19 and 22	(Blaschek et al., 2011)
Percentage of living spermatozoa after thawing	Holstein	23 and 27	(Druet et al., 2009)
Percentage of living spermatozoa after thawing	Holstein	5, 7, 8, 10, 17, and 23	(Michot, et al., unpublished)
Motility	Holstein	7, 11 et 27	(Druet et al., 2009)
Motility	Holstein	4, 10, 11, 13 and 27	(Michot, et al., unpublished)
Sperm volume	Holstein	15 and 22	(Druet et al., 2009)
Sperm volume	Holstein	3, 14, 15, 17, 22, 23 and 28	(Michot, et al., unpublished)
Sperm concentration	Holstein	1, 3, 9, 11, 13, 21 and 28	(Michot, et al., unpublished)
Number of spermatozoa	Holstein	3, 12, 21, 28	(Michot, et al., unpublished)

### Oocyte quality

Oocyte quality is crucial for conception rates and early embryonic survival, relying on the precise control of maternal factors that have been accumulated during oocyte growth as well as maturation conditions. Moreover, there is a large amount of evidence supporting the role of the oocyte in regulating follicle growth and development, and thereby its own development, by the production of oocyte growth factors acting on supporting granulosa cells. In addition, intensive bi-directional exchanges of nutritive and signaling molecules occur inside of the cumulus-oocyte complex (COC) during oocyte maturation. Gene expression studies performed to compare oocytes before and after maturation as well as cumulus cells (CC) surrounding immature and mature oocytes (Regassa et al., 2011; Charlier et al., 2012b) from cows carrying either favorable “Fertil+” or unfavorable “Fertil-” haplotype from a QTL located on BTA3 (Coyral-Castel et al., 2012; Brisard et al., 2014) highlighted genes involved in energy metabolism. Gene expression was also compared between COC collected from repeat breeder and normally fertile Holstein heifers, showing 43 upregulated and 135 downregulated genes in repeat breeder heifers, mainly involved in metabolism, substrate/ion transport, cell adhesion, and cytoskeleton (Puglisi et al., 2013).

Characterization of maternal transcript expression profiles for in vitro or in vivo oocyte maturation as a model of differential developmental competence revealed a variety of maternal transcripts including *BCAR4*, as a novel maternal effect gene (Angulo et al., 2013), thereby highlighting the role of post-transcriptional regulation during oocyte maturation (Thelie et al., 2007). Gene expression profiles of oocytes derived from aged cows were shown to differ from those of oocytes derived from young cows, pointing to an oxidative phosphorylation process and mitochondrial dysfunction (Takeo et al., 2013). Furthermore, sets of miRNA have been identified in bovine oocytes or cumulus cells, showing specific expression dynamics during oocyte maturation (Miles et al., 2012; Abd El Naby et al., 2013). By defining the gene expression profile associated with oocytes quality, these studies pave the way for future robust gamete quality assessment.

### Focusing on Embryo and Fetus Development Capabilities

In recent years, high-throughput and epidemiologic studies have demonstrated the major importance of early embryonic mortality and fetus lethality in pregnancy failures (Diskin and Morris, 2008; Humblot et al., 2009; Kropp et al., 2014a). Thus, research has focused on major determinants of embryo and fetus development capabilities, such as embryonic genome activation, blastocyst formation, conceptus elongation, embryonic disc dif-

ferentiation (or gastrulation), implantation, fetus development, as well as maternal functions (early uterus cross-talk with the embryo, placentation).

### Exploring genetic variability to discover key players

A QTL fine mapping study, based on large pedigrees and improved phenotyping procedures, identified three QTL for non-fertilization or early embryo mortality rate [chr 2 (8Mb); 3 (24Mb); 6 (95Mb)], three for late embryonic mortality rate [chr 1 (8Mb); 3 (22Mb); 6 (92Mb)], eight for total embryonic mortality [chr 1 (6Mb); 1 (8Mb); 3 (22Mb); 4 (66Mb); 6 (92Mb); 6 (95Mb); 18 (49Mb); 26 (41Mb)], two for fetal mortality [chr 14 (31Mb); 26 (35Mb)], and three for abortions [chr 3 (23Mb); 18 (49Mb); 26 (40Mb)] (Lefebvre et al., 2011). In addition, recessive genetic defects may be responsible for reduced fertility through prenatal lethality. Genomic regions containing lethal mutations affecting in utero development were identified in Ayrshires, Brown Swiss, Holstein, Jersey, Montbéliarde, and Normande breeds (Table 4). Altogether, a significant negative effect on calving rate was observed for 20 haplotypes, confirming their association with in utero lethal mutations. Six regions were further investigated using whole-genome sequencing data from heterozygous bull carriers and strong candidate causative mutations were detected in *FANCI* (Brachyspina), *APAF1* (haplotype H1), *SMC2* (HH3), *GART* (HH4), *CWC15* (JH1), and *SLC37A2* (MH1) genes. In Nordic Red cattle, a 660-kb deletion on BTA12 was shown to lead to embryonic lethality, probably due to the loss of *RNASEH2B*. Interestingly, this deletion was also shown to increase milk yield, explaining why several Red Cattle populations included about 25% carriers despite the gene’s effect on fertility. This study evidenced that structural variants may contribute to phenotypic variation, and that balancing selection might be more common in livestock species than previously thought.

High-throughput SNP genotyping and whole-genome sequencing combined with large databases of performance records and observatories dedicated to the detection of genetic defects now offer unprecedented opportunities to accelerate the dissection of the genetic architecture of fertility and embryo development. This will improve breeding tools and herd management, as well as the general knowledge of the function of mammalian genes.

### Omics of embryo development capabilities

Biotechnologies such as ovum pickup and in vitro fertilization (OPU-IVF), in vivo embryo collection, embryo genotyping, as well as primary cultures of endometrium and oviduct cells have expanded the range of Omics measures available to explore biological functions and pathways involved in embryo development. Numerous transcriptome studies have identified sets of genes related to key developmental periods: i) during maternal-to-embryonic transition (MET) when maternal RNA and pro-

**Table 4. Haplotypes and genes associated with prenatal death in cattle.**

Breed	Haplotype	Chromo	Region (Mb)	Gene name	Reference
Ayrshires	AH1	17	65.9-66.2		(Cooper et al., 2014)
Brown Swiss	BH1	7	42.8-47.0		(VanRaden et al., 2011)
	BH2	19	10.1-11.1		(Schwarzenbacher et al., 2012)
Finnish Ayrshire and Swedish Red		12	20.1-20.7	RNASEH2B	(Kadri et al., 2014)
Holstein	HH0	21	21.1-21.2	FANCI	(Agerholm et al., 2006) (Charlier et al., 2012) (Sahana et al., 2013)
	HH1	5	63.1	APAF1	(Adams et al., 2012) (VanRaden et al., 2011)
	HH2	1	94.8-96.6		(VanRaden et al., 2011)
	HH3	8	95.4	SMC2	(VanRaden et al., 2011) (Sahana et al., 2013)
	HH4	1	1.3	GART	(Fritz et al., 2013)
		5	66.8-68.6		(Sahana et al., 2013)
		5	106.7-114.4		(Sahana et al., 2013)
		7	33.3-34.6		(Sahana et al., 2013)
		11	60-63		(Sahana et al., 2013)
		19	131-144		(Sahana et al., 2013)
Jersey	JH1	15	15.7	CWC15	(Sonstegard et al., 2013; VanRaden et al., 2011)
Montbéliarde	MH1	19	27.6-29.4		(Fritz et al., 2013)
	MH2	29	27.9-29.1	SLC37A2	(Fritz et al., 2013)
Normande	NH1	24	38.1-39.2		(Fritz et al., 2013)
	NH2	1	45.7-146.8		(Fritz et al., 2013)
	NH5	7	3.6-4.6		(Fritz et al., 2013)
	NH6	15	59.8-61.1		(Fritz et al., 2013)

teins stored in the oocyte are gradually degraded and transcription of the embryonic genome is activated; ii) between the time of hatching of the blastocyst and its subsequent elongation (Day 8 to 17); iii) and at initiation of implantation on Day 19.

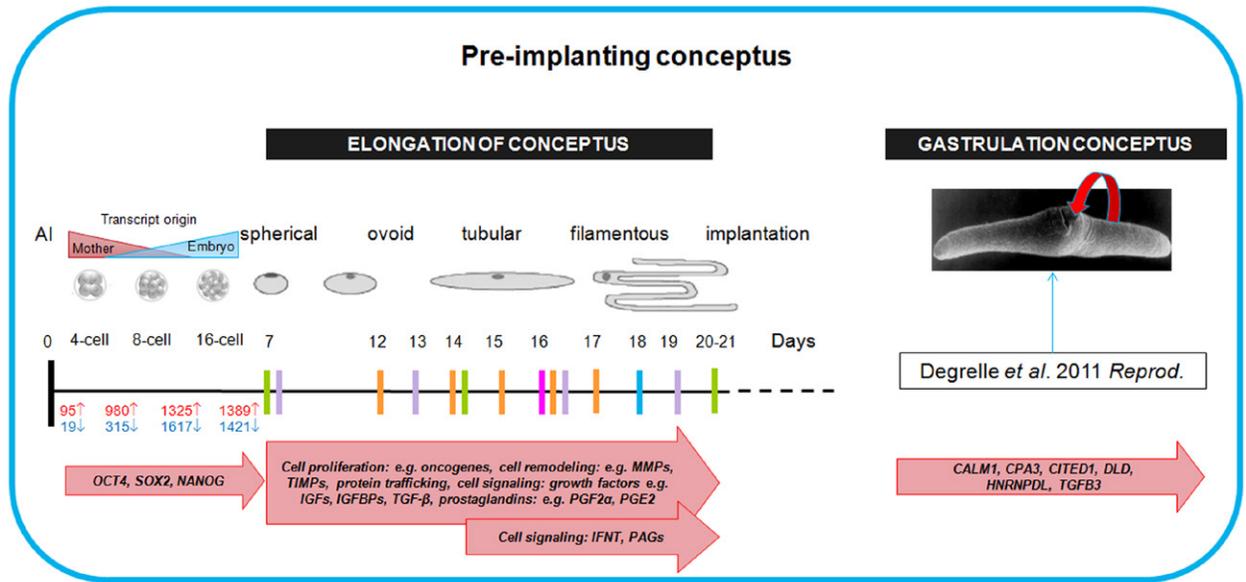
First, comparisons of transcriptome profiles were developed to assess mechanisms regulating the onset of embryonic genome activation (EGA; review in Graf et al., 2014a). Although mechanisms regulating EGA are broadly conserved in mammals, OCT4, SOX2, and NANOG proteins were not detected during the pre-EGA phase in cattle, suggesting that they are not essential for bovine EGA in contrast to the mouse (Khan et al., 2012). A role of specific miRNA in MET was also suggested for bovine embryos (Mondou et al., 2012). Very recently, RNA sequencing of metaphase II oocytes and 4-, 8-, 16-cell, and blastocyst stage embryos established the most comprehensive transcriptome data set of bovine early development (Graf et al., 2014b). Genes activated at the 4-cell stage were related to RNA processing, translation, and transport, preparing the embryo for major EGA. At the 8-cell stage up to the 16-cell stages, genes from a broad range of functional categories were found to be activated, including transcription and translation as well as protein ubiquitination while genes activated in blastocysts included regulators of early lineage specification.

Genomics was also applied to predict embryo development capabilities and search for biomarkers. For instance, transcriptional profiles from biopsies of in vitro-produced Day 7 blastocysts were related to pregnancy success after in vivo transfer to recipient females (El-Sayed et al., 2006). These results are, however, difficult to interpret due to the sensor/driver properties of the uterus that may influence the “intrinsic” competence of the blastocyst (Sandra et al., 2011). Although not used much, assessing

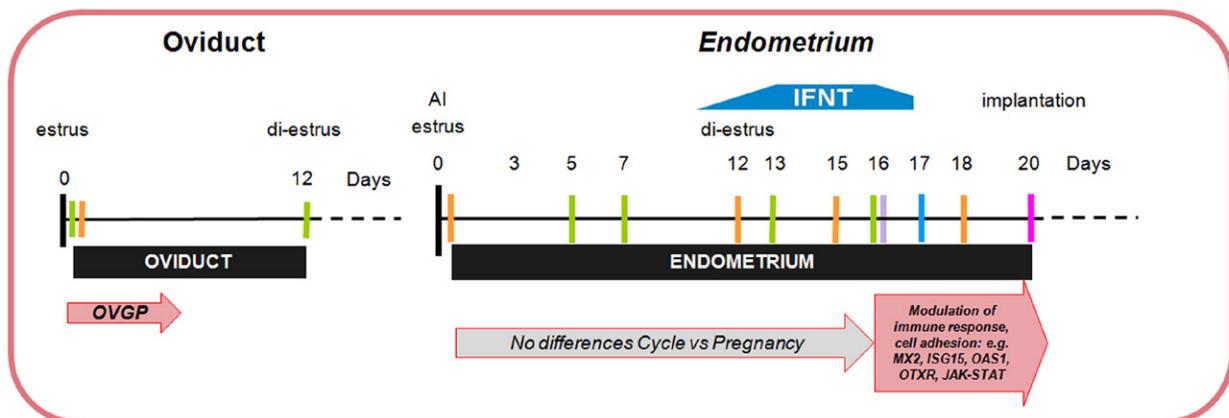
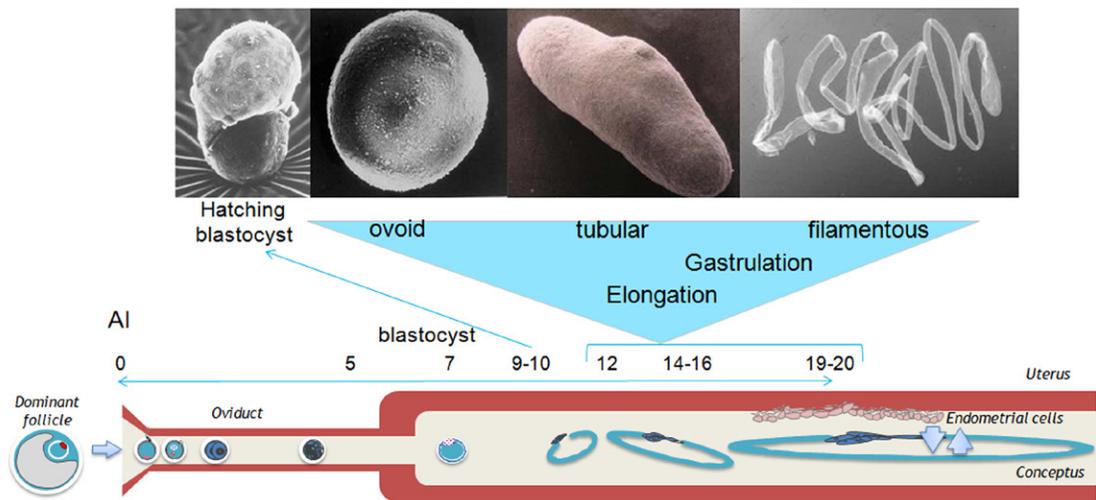
embryo development through its elongating capabilities is a less expensive way to account for Day 7 quality after transfer (Berg et al., 2010). In addition, higher expression of miR-25, miR-302c, miR-196a2, and miR-181a was observed in embryos that failed to develop from the morula to blastocyst stage compared with those that developed to the blastocyst stage. Interestingly, these miRNA were also found to be secreted into the embryo culture media (Kropp et al., 2014b).

Gene expression analysis of elongating and gastrulating embryos preceded several studies (review in Hue et al., 2007; Hue et al., 2012). Comparison of Day 7 and Day 14 blastocysts highlighted 706 differentially expressed genes, including tumor suppressor genes, oncogenes, and genes controlling cell cycle and apoptosis (Ushizawa et al., 2004), and one third of transcripts from an ovoid stage library were associated with cell cycle and proliferation (Degrelle et al., 2005). RNA sequencing of bovine conceptus at five key stages of the critical window of pre- and peri-implantation development (Day 7, 10, 13, 16, and 19) revealed a total of 5,500 differentially regulated genes clustered into nine gene expression patterns forming sequential transcript dynamics across these developmental stages (Mamo et al., 2011a).

There are currently only a few studies in cattle describing the establishment of anterior-posterior and dorsal-ventral axes, which are essential for the formation of a viable conceptus. Genes identified from a transcriptome analysis of extra-embryonic tissues (Degrelle et al., 2011) provided a first view on the possible link, in cattle as well as in the mouse, between gene expression in extra-embryonic tissues and embryonic differentiation. The challenge remains to evaluate in vivo embryo development, taking into account components from the “maternal environment” together with the “intrinsic quality of the embryo.”



Pictures M. Guillomot



**Figure 2.** Major functions and genes involved in conceptus development and maternal support and recognition of pregnancy (transcriptomics). Only a few genes are known to have an effect on fertility in the oviduct. No differences between estrus cycle and pregnancy were observed in the endometrium whereas modulation of immune response, cell adhesion, and transduction of signal are active processes after Day 16. Progesterone secretion is similar between estrus cycle and pregnancy until Day 16 but IFNT is secreted as soon as Day 10 with a maximal amount between Day 13 and 16 and impact endometrial genes expression during the elongation phase of the bovine conceptus. Many clusters of genes are expressed within the conceptus during development. Figure 2 is based on studies investigating pre-implanting conceptus (Ushizawa et al., 2004; Degrelle et al., 2005; Degrelle et al., 2011; Mamo et al., 2011b; Degrelle et al., 2012; Betsha et al., 2013; Graf et al., 2014b), the role of the oviduct (Bauersachs et al., 2004); and the role of the endometrium (Bauersachs et al., 2005, 2006, 2009, 2012; Mitko et al., 2008; Mansouri-Attia et al., 2009b; Mansouri-Attia et al., 2009a; Walker et al., 2010; Forde et al., 2011; Mamo et al., 2012a).

## Omics to Explore the Role of Maternal Environment in Fertilization and Embryo Development

Interactions with the maternal environment are crucial for fertilization and embryo development. Indeed, some proteins from the oviduct interact with oocyte and sperm before fertilization while key events of early embryo development occur in the oviduct as well. Likewise, the uterus plays a key role to support embryo development, and conceptus-derived factors such as interferon-tau (IFNT) induce or further amplify expression of a large number of genes in the endometrium of pregnant animals.

### **Oviduct**

The oviduct has a pivotal role during early pregnancy, supporting both fertilization and the development of a healthy embryo (Besenfelder et al., 2012), as exemplified by the suboptimal *in vitro* embryo development. Identifying the oviductal secretome may be a way to improve *in vitro* methods. For example, a bovine transcriptomic study revealed 77 transcripts differentially expressed in the oviduct between estrus and di-estrus (Bauersachs et al., 2004). One transcript, oviductal glycoprotein (OVGP), depends on estrogen and plays a positive role in capacitation and sperm motility, thus having a role in the interaction between gametes and fertilization (Buhi, 2002). Other genes involved in protein secretion, stress response, intracellular signaling, growth, proliferation, and cell differentiation were also highlighted. One study evaluated gene expression changes in bovine oviduct epithelial cells (BOEC) in the presence of developing bovine embryos (8 d cultured embryos). This study identified 34 upregulated genes due to the presence of embryos, including genes known to be induced by interferons and playing a major role in antiviral and immune responses (Schmaltz-Panneau et al., 2014). This study suggested thus that embryo signaling during early pregnancy may trigger changes in the gene expression program of oviductal cells. In addition, gene expression in the oviduct was shown to be influenced by a mild postpartum undernutrition (Valour et al., 2013). Collectively, these results prompt the need for deciphering physiological and pathological oviductal mechanisms during early pregnancy in cattle, especially in postpartum dairy cows (Rizos et al., 2010).

### **Uterus and cross-talk at implantation**

Finely tuned spatiotemporal regulations of gene expression in the endometrium are required to drive conceptus elongation and establish uterine receptivity to implantation. The two main modulators of these processes are progesterone and IFNT. Many transcriptome studies have documented changes in gene expression from the time of maternal recognition of pregnancy to implantation (review in Forde and Lonergan, 2012; Bauersachs and Wolf, 2013). Changes in the endometrial transcriptome are independent of the conceptus until high synthesis of IFNT by the conceptus induces maternal recognition of pregnancy (Forde et al., 2011) and initiation of immune tolerance for implantation (Walker et al., 2010). Interestingly, changes in endometrium gene expression at implantation were observed (Mansouri-Attia et al., 2009a), depending on conceptus origin (artificial insemination, *in vitro* fertilization, or somatic cell nuclear transfer). Since a higher rate of pregnancy losses have been observed during the implantation period for embryos produced *in vitro* (Heyman, 2005), these results emphasize both the differences in embryo quality associated with biotechnologies and the role of conceptus-uterus cross-talk in successful embryo implantation and pregnancy. This has also raised

questions about the impact of culture conditions on embryo development, and a review was published recently to summarize transcriptome data, focusing on the impact of suboptimal preimplantation culture conditions on pre-, peri-, and post-implantation embryos (Salilew-Wondim et al., 2014).

RNA sequencing was performed to study the transcriptome of bovine conceptus at five key stages (Days 7, 10, 13, 16, and 19) and identified 236 and 1,409 upregulated genes involved in maternal recognition and initiation of implantation, respectively (Mamo et al., 2011a). In addition, a comprehensive list of molecules potentially secreted from the conceptus and which interact with receptors on the endometrium (133 genes) and vice-versa (121 genes) was determined for the critical window of maternal recognition of pregnancy (Mamo et al., 2012b). Some of these may prove to be good early biomarkers of pregnancy (Figure 2).

### **Dam physiological status**

Changes in gene expression related to dam physiological status appeared to affect Day 18 conceptus metabolism, especially when comparing heifers and lactating cows. Thus, despite a similar morphological development, many biosynthetic pathways appeared to be more active in embryos from heifers than from cows, suggesting that the former seemed healthier and were in an environment more conducive to proper development (Valour et al., 2014).

Fatty acid supplementation in the diet is thought to influence reproductive performance, depending on the fat type given. In particular, omega-6 and omega-3 polyunsaturated fatty acids act on follicular growth, steroid synthesis, and prostaglandin metabolism in the ovary and endometrium, respectively. Some *in vitro* studies have been performed to assess the direct effects of fatty acids on oocyte and early embryo development (review in Leroy et al., 2014) but generated somewhat conflicting results. However, emerging Omics data suggest that the phenotype of the early embryo can be dramatically altered by exposure to fatty acids during final oocyte maturation. In particular, dietary-induced hyperlipidaemic conditions can be harmful for embryo development and metabolism, leading to a glucose-intolerant state.

## Perspectives and Conclusions

The physiology of reproduction in cattle is a highly dynamic and complex system sensitive to factors such as genotype, nutrition, or lactation. Together with refined phenotyping procedures, the advent of Omics and GWAS methods has provided the potential to screen animals for biomarkers associated with reproduction and has brought some insight into key genes or pathways. However, these results have often failed to establish clear links between the genome and final phenotypes. A first step toward data integration would be to search for polymorphisms in or near candidate genes identified by functional studies and test for association with fertility and other traits. Cochran and colleagues recently performed such a work starting from a set of 434 candidate SNP. Among them, about 100 SNP could be statistically analyzed, showing 40 SNP significantly associated with daughter pregnancy rate (Cochran et al., 2013a), as well as nine with cleavage rate and 12 with percentage of blastocysts obtained after *in vitro* fertilization (Cochran et al., 2013b). Interestingly, only a few SNP were found to have an antagonistic relationship between fertility and milk yield, suggesting that the selection for fertility is possible without adverse effect on production. Thanks to the wealth of sequencing data generated by the 1,000 bull genomes project (Dae-twyler et al., 2014), it's likely that the list of candidate SNP will grow up in a

near future, providing a way to improve the accuracy of genomic estimates. However, identifying causative SNP in genes controlling reproduction will undoubtedly be insufficient, as exemplified by the mutation in the CWC15 housekeeping gene, which has been associated with infertility in Jerseys (JH1 haplotype). Furthermore, whether phenotypic variations result from large effects of rare mutations or from small effects of many mutations that affect gene expression or function is thus still under debate.

Moreover, an integrated and translational systems biology modeling approach may be an effective way of tackling such a complex problem (Loor et al., 2013; McNamara and Shields, 2013). Bioinformatics approaches still need to be refined to enable interpretation of such a large amount of data although a number of mathematical models have been developed to help in the reconstruction of networks in model organisms.

New biomarkers emerging from these Omics studies may supplement traditional reproductive phenotypes to evaluate reproductive potential and gamete quality and predict pregnancy success. In particular, *in vitro* approaches to assess embryo intrinsic quality or standardized “adaptative” challenges (e.g., response to stressors or contrasted nutrition strategies) are still lacking but would allow further study of interactions between the genetic background, the maternal environment, and their impact on fertility. Ideally, non-invasive methods such as prediction of embryo development capabilities and prediction of uterus receptivity should be developed to assess subsequent embryo development.

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## About the Authors



Damien Valour is an agricultural engineer and PhD from AgroParisTech, one of the foremost French Graduate Institutes in Science and Engineering. His thesis research project on embryonic development was conducted at INRA, within the Developmental Biology and Reproduction Joint Research Unit (UMR 1198). He especially studied transcriptome changes in the bovine embryo during the preimplantation period, establishing relationships with maternal postpartum metabolic and hormonal profiles and providing new insights in fundamental reproductive biology. This led him to acquire skills in wet-lab “Omics” tools as well as in integrative and translational approaches, developing new computational tools to analyze high throughput biological data in nutrigenomics. He’s appointed by UNCEIA, the professional organization federating French breeding and artificial insemination cooperatives to integrate cattle Omics data collected over years in the course of several research projects on female fertility and embryo development.



After having worked 15 years at INRA on ruminants and horses genomics, Laurent Schibler has joined UNCEIA in 2013 to manage the R&D team. As an agricultural engineer and PhD in molecular genetics and structural genomics, he forged his career at the crossroad of genetics and physiology. He firstly developed basic research to set up innovative positional cloning strategies and contributed to the identification of several QTLs and genes of agronomical interest. Then, he used state of the art genomics and proteomics technologies to study cartilage maturation in several transgenic mouse models and bring new insights into molecular mechanisms involved in equine osteochondrosis. Currently, he aims to develop integrated projects in cattle reproduction, combining genetics, genomics and physiology.

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