Invited Article

GENOMIC REGULATION OF ESTROUS BEHAVIOR IN DAIRY COWS

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ABSTRACT

The expression of estrous behavior by dairy cows has progressively declined over the past several decades. Reduced estrus expression by cows is one of the factors insinuated as the cause for suboptimal fertility. Variation in the expression of estrous behavior within and between cows is associated with alterations in hormone profile particularly circulating steroids, progesterone (P4) and estrogen (E2), concentrations prior to and during estrus. Estradiol acts on the brain by genomic, non-genomic and growth factor-dependent mechanisms to elicit estrus behavior. Progesterone plays a pivotal priming role for full display of estrus. A number of genes expressions were associated with duration and intensity of estrous behavior. A better understanding the gene expression in the hypothalamic-pituitary and ovarian axis in follicular and luteal phase cows, respectively, is critical. This could contribute to improved genomic selection strategies for appropriate expression of estrus behavior.

INTRODUCTION

Reproductive performance of high-yielding dairy cows has undergone a major decline over the past five decades. Dairy cattle selection for higher milk yield has coincided with a decline in fertility (Butler, 2003; Figure 1). The genetic correlation between milk yield and female fertility, although antagonistic, is moderate in magnitude from 0.2 to 0.6 (Pryce et al., 2004; Bello et al., 2012). However, in recent years, the causal physiological mechanisms responsible for this decline have begun to be unraveled. It is apparent that poor genetic merit for fertility traits is associated with multiple defects across a range of organs and tissues that are antagonistic to achieving satisfactory fertility performance. The principal causes include disproportionate mobilization of body energy reserves, unfavorable metabolic status, delayed resumption of cyclicity, increased incidence of uterine disease, dysfunctional estrus expression and insufficient luteal phase progesterone concentrations (Table 2; Cummins et al., 2012; LeBlanc 2013). Similarly, the proportion of dairy cows exhibiting standing estrus has declined from 80 to 50% and the average duration of true estrus has progressively decreased from 18 h to 7 h (Dransfield et al., 1998; Dobson et al., 2008). In addition, the intensity with which secondary behavioral signs of estrus are displayed have also steadily declined, even to the level that some cows show no estrus signs at all ('silent' estrus). The reduced duration and intensity, or even the absence, of estrous behavior makes it difficult for dairy farmers to detect cows in estrus, consequently, estrus may go undetected, or weak secondary estrous signs may be misinterpreted as 'estrus'.

Estrus or "heat" is a period during the reproductive cycle when female animals become sexually receptive for mating. A cow standing to be mounted by herd-mates is the most accurate behavioral sign of estrus. In order for standing

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behavior to be expressed, cattle must be allowed to interact which is a concern in the modern intensive management practices. Secondary behavioral signs that are exhibited prior to standing heat include: trying to mount other animals not in heat, increased urination, isolation from the herd, and social behaviors such as laying her head upon the backs of other animals (chinning). A cow may show signs of nervousness and restlessness such as walking, bawling, and decreased milk production from less time spent eating. Physical signs that indicate estrus include the vulva becoming red and swollen and excess mucus discharge. Only a cow in estrus, when mounted, will show an immobilization response (Figure 2). This is the only behavioral sign for true estrus and is considered as the primary sign of estrus. All other behavioral signs of estrus as well as the physical signs are useful to identify cows that are suspected to be in estrus but are not conclusive.

Estrus expression is caused by E2 being produced within developing ovarian follicles on the ovary. Efficient and lucrative reproductive performance of a dairy herd requires routine but assiduous heat detection and proper timing of artificial insemination. If behavioral or physical signs are not obvious, estrus may even pass unnoticed. Successful recognition of the signs of estrus for mating can result in increased conception rates. Failure to detect heat per se is a major factor contributing to low fertility. Approximately half of the heats are undetected on dairy farms (error of omission) and based on levels of the milk P4 up to 15% percent of the cattle presented for insemination are not in heat (error of commission). These two errors result in economic loss for the producers because of extended calving intervals and additional semen expense.

Challenges of estrus detection on farms include: cows with anovular conditions (Wiltbank et al., 2002); attenuation of the duration of estrus behavior associated with increased milk production near the time of estrus, resulting in shorter periods of time in which to visually detect estrus behavior (Lopez et al., 2004); few cows expressing standing estrus at any given time (Roelofs et al., 2005; Palmer et al., 2010); silent ovulations (Palmer et al., 2010; Ranasinghe et al., 2010; Valenza et al., 2012); and reduced expression of estrus owing to confinement housing systems (Palmer et al., 2010) with concrete flooring (Britt et al., 1986). It should be noted that genetic merit for fertility traits had a significant effect on characteristics of the estrous cycle.

Understanding the contextual individual differences in duration, and especially intensity of estrus behavior, may lead to opportunities to improve estrus expression and reduce the group of cows in which intensity and duration of estrus behavior are too low for adequate and efficient heat detection. This may increase in fertility. This review emphasizes selected aspects of the physiological, neurophysiological and central genomic background of variation in the expression of estrus behavior.

Ovarian hormones and estrous behavior

Sexual stimulation is dependent on neural (sensory and cognitive), hormonal and genetic factors. It activates cognitive and physiological processes that can eventually lead to sexual behavior (Schober and Pfaff, 2007). Regarding hormonal factors, sexual arousal in female animals is dependent on E2 and P4. During the estrous cycle, the sequential actions of the ovarian steroid hormones P4 and E2 in the central nervous system (CNS) cause cyclical fluctuations in the degree of sexual excitement and facilitate expression of estrous behavior just before ovulation.

Estrogen plays a key role in the regulation of endocrine and behavioral events associated with the estrous cycle. Apart from estrogens' central role in triggering the gonadotropin surge and ovulation, it facilitates the estrous behavior,

and thus E2 indirectly synchronizes mating and ovulation. The patterns of GnRH synthesis and pulsatile release from the hypothalamus are mainly regulated by E2 and P4. E2 stimulates luteinizing hormone (LH) synthesis, but at levels below a certain threshold value it inhibits the release of LH. Above this threshold, the inhibitory effect on LH release switches to a stimulatory effect, which results in the LH surge and ovulation. The shift from inhibition to stimulation may be dependent on the site of action of E2, that is, a switch from acute membrane signaling to classic genomic signaling (Arreguin- Arevalo and Nett, 2006). The LH surge is driven by an increased pituitary responsiveness to gonadotropin releasing (GnRH), which is determined by the amount of GnRH receptors (GnRHRs) expressed on gonadotropes. Pulsatile GnRH release, facilitated by high E2 concentrations during the preovulatory period, elevates GnRHR gene expression, whereas high P4 concentrations in the luteal phase inhibit the GnRHR gene expression.

The effect of estradiol on estrous behavior in dairy cows is 'all or none'. Estrous behavior is induced as soon as a threshold of estradiol concentration is reached and additional estradiol above this threshold will not further enhance the duration and intensity of behavioral estrus expression (Allrich, 1994). However, several studies with modern high producing dairy cows indicate a relatively strong positive relationship between the estradiol blood concentration and the duration and intensity of behavioral estrus during the late follicular phase. Regarding the role of P4 in the expression of behavioral estrus in ruminants, there is much evidence that suggests an important facilitator role of P4 priming. This role includes an increase in the estradiol responsiveness of specific areas of the central nervous system (CNS; especially the hypothalamus) involved in the expression of behavioral estrus.

Central role of E2 in regulation of estrous behavior

Estradiol plays a pivotal role in the induction of estrous behavior (Pfaff, 2005). It has a self-amplifying effect as it stimulates the expression of E2 receptors (ERs) in the brain. The duration of estrous behavior was found to depend mostly on the duration of E2 presence rather than on its maximum concentration (Fabre-Nys et al., 1993). The effects of E2 are highly similar in different species, although threshold concentrations for the induction of estrous behavior may vary between animal species. Lower E2 concentrations are required for expression of estrous behavior than required for the LH surge and that estrous behavior can be induced independently of the LH surge. Estrous behavior and the LH surge can be separated by experimental reduction of E2 levels and stress (Dobson et al., 2008). The observations suggest that stress reduces P4 exposure before estrus and/or E2 production by the dominant follicle and thereby reduces expression of estrous behavior (Dobson et al., 2008). In cows and other domestic ruminants, the behavioral expression of estrus is preceded by a luteal phase of 12 to 15 days during which P4 concentrations are high (Fabre-Nys and Gelez, 2007). High P4 concentrations during the luteal phase inhibit the E2-induced gonadotropin surge by reducing pituitary responsiveness to GnRH (Attardi et al., 2007; Richter et al., 2005). The duration of P4 presence and the P4 amplitude in the luteal phase influence the time interval between rise in E2 levels and the induction of estrous behavior and the LH surge, probably by affecting the neural mechanisms that are involved in GnRH release (Skinner et al., 2000). The exact functions of P4 in the priming as well as the inhibition of estrous behavior are debated (Zalányi, 2001; Weiss et al., 2006; Attardi et al., 2007) and seem to differ between species (Fabre-Nys and Gelez, 2007).

Estrogen signaling and gene expression in the brain in female sexual behavior

Estrogen acts in the brain by a variety of genomic, non-genomic and growth factor-dependent mechanisms (Cardona-Gómez et al., 2003; Vasudevan et al., 2005; Kelly and Qiu, 2010) (Figure 3).

1. As far as the genomic mechanisms are concerned, estradiol regulates transcription of genes in the neurons and glia by the activation of classic nuclear E2 receptors (ERs). The two ERs, ERα and ERβ, are products of different genes. The expression and distribution of ERβ in the brain of cattle has not yet been studied. In addition, only limited information is available about the expression and distribution of ERα. The typical mode of genomic action of estradiol through stimulation of ERs is complemented by alternative non-genomic actions.
2. In regards to non-genomic action, estradiol acts at the membrane or in the cytoplasm of neurons. These non-genomic actions of estradiol are linked to the activation of different kinases, cyclic adenosine monophosphates (cAMP) and intracellular calcium (Ca2 +), and activation of other transcriptional regulators such as cAMP response element-binding protein (Cardona-Gómez et al., 2003; Vasudevan et al., 2005; Kelly and Qiu, 2010).
3. The effects of estradiol in the brain may also be mediated by the activation of growth factor signaling (Cardona-Gómez et al., 2003). Molecular interactions include reciprocal regulation of ERs and insulin like growth factor-I receptors (IGF-IRs), adrenergic α-1 receptor expression and activation, as well as Mitogen-activated protein kinases (MAPK) and phosphatidylinositol-3-kinases/ Protein kinase B (PI3K/Akt) signaling pathway activation. Functional interactions between IGF and ER pathways affect axonal and dendritic growth, estrous cycle associated synaptic plasticity, gonadotropin release/ovulation, and reproductive behavior/sexual receptivity.

Estrogen signaling and gene expression in the brain in relation to the expression of estrous behavior in dairy cows

Sexual behavior in cows

The gene expression studies revealed several E2 induced mechanisms that are involved in arousal, more specifically increase in activity such as standing, locomotion and exploratory behavior in cows (Roelofs et al., 2005a). Arousal, a general activation of brain and behavior, precedes the "standing" response and results from signaling by neurotransmitters like norepinephrine. In the brain, the expression of ERα and its downstream effects are essential for arousal. E2-induced down-regulation of prostaglandin-D synthase (PTGDS) in the preoptic area (POA) increases arousal response and PTGDS downregulation is also associated with "standing" response (Pfaff et al., 2008). The initial step in the induction of sexual behavior is the E2 controlled alteration of neuronal activity in the ventromedial nucleus (VMN). Estrogen priming alters gene expression in VMN neurons, resulting in the activation of a variety of neurotransmitters and neuropeptides. For example, E2 induces expression of adrenergic receptor genes in the VMN and increases the proportion of neurons that respond to stimulation of adrenergic receptors, which is the first step of a signal transduction pathway resulting in standing" behavior. Another example is the E2-induced expression of glial specific genes, including glutamine synthetase, in the arcuate nucleus (ARC) and VMN nuclei, and in the amygdala and hippocampus, thus facilitating the glutamatergic neurotransmission important for estrous behavior. Figure 4 illustrates miRNA and associated genes involved in regulation of estrus; miR18B, miR188, miR202, miR219, miR222, miR223, miR378, miR422A, mir422B, and miR448 were regulating genes responsible for hormonal regulation of sexual behavior.

Genomic regulation of estrous behavior and associated genes in brain

Relatively little is known about the genomic regulation of estrous behavior in dairy cows. However, in a recent series of studies, gene expression in the anterior pituitary and four brain areas (amygdala, hippocampus, dorsal hypothalamus and ventral hypothalamus) in estrous and luteal phase cows, respectively, has been measured, and the relation with estrous behavior of these cows was analyzed (Kommadath et al., 2010, 2011 and 2013; Kommadath, 2012). The estrous behavior recorded in these cows was quantified as heat scores (estrous behavior) according to the method of Van Eerdenburg et al. (1996), and the scores from multiple consecutive cycles were averaged to obtain the average heat score per cow. In these studies (Kommadath et al., 2010, 2011 and 2013; Kommadath, 2012), a number of genes or gene clusters were found to have expression levels associated with the heat score.

Table 1 showed estrous behavior-associated genes and processes in dairy cows grouped in the GAPPS modules described for female sexual behavior [Based on the research by Pfaff and co-workers on lordosis behavior in rodents, it has been proposed that estradiol-activated genes in the VMH are organized in five modules referred to as GAPPS modules (Mong and Pfaff, 2004; Pfaff, 2005). GAPPS stands for: Growth of hypothalamic neurons; Amplification of the E2 effects by P4; Preparative behaviors; Permissive actions on the sex behavior circuitry; and Synchronization of mating behavior with ovulation].

Growth: First module is the increase in the input/output connections for behaviour-directing hypothalamic neurons or synaptic plasticity): Several of the genes found associated with the heat score in cattle are related to synaptic plasticity (Kommadath et al., 2010, 2011). These genes can therefore be considered to be part of the Growth module, which is characterized by estradiol-dependent outgrowth of hypothalamic neurons. This growth may also apply to amygdala and hippocampus, and the anterior pituitary, as genes related to synaptic plasticity were not limited to the hypothalamic area. Analysis of gene co-expression networks between the brain areas and anterior pituitary (Kommadath et al., 2013) revealed gene clusters that correlated with estrous behavior, of which a number of hub genes have been reported to have functions related to neuronal growth or plasticity. Furthermore, several ribosomal genes associated with estrous behavior in the association studies (Kommadath et al., 2011) or the co-expression studies (Kommadath et al., 2013) across several brain areas.

Amplification: The second module is the amplification of the E2 effect by P4, mediated by the nuclear progesterone receptor (PGR). In cattle, the PGR expression in the anterior pituitary was observed to be upregulated on day 0 (estrous phase) compared with day 12 (luteal phase) of the estrous cycle (Kommadath, 2012). The expression of PGR was not found to be related with the heat score of the cows. Furthermore, in cattle, P4 levels in cows remain low during estrus. Nevertheless, an increased expression of PGR on day 0 could suggest a relation of the PGR with estrous behavior, perhaps via ligand-independent pathways (Mani and Blaustein, 2012).

Preparation: The third module is preparation for mating. The expression of oxytocin and arginine vasopressin genes in several brain areas of cows was associated with estrous behavior (Kommadath et al., 2010 and 2011). Oxytocin, produced by the supraoptic and paraventricular nuclei of the hypothalamus, is released within the brain where it acts on specific oxytocin receptors to elicit effects such as female sexual receptivity, grooming behavior and partner bonding (Leng et al., 2008). In the presence of E2, oxytocin exerts an anxiolytic effect, mediated by increases in oxytocin-binding density in the lateral septum, thereby favoring courtship and mating (McCarthy et al., 1997; Mong and Pfaff, 2004). Similar to oxytocin, vasopressin is associated with sexual

behavior and bonding and its expression is under the control of E2 and P4 (Patisaul et al., 2003; Kalamatianos et al., 2004; Curley and Keverne, 2005; Donaldson and Young, 2008). Genes that may also be grouped in the Preparation module are pro-opiomelanocortin (POMC), melanin-concentrating hormone receptor (MCHR)1, cholecystokinin (CCK), dopamine receptor D2 (DRD2), hydroxytryptamine receptor 2A (HTR2A) and gamma-aminobutyric acid receptor subunit alpha-6 (GABRA6), whose expression levels in at least one of the brain areas are associated with estrous behavior score (Kommadath et al., 2011). These genes are known to modulate emotional states such as anxiety and satiety (Rex et al., 1997; Marsh et al., 2002; Uhart et al., 2004; Millington, 2007). The link between fertility and appetite is evident from the finding of the POMC and MCHR1 genes, both of which play roles in feeding behavior, metabolic rate and feed intake (Marsh et al., 2002; Millington, 2007). It is known that interactions between monoamines (dopamine, serotonin, noradrenaline) and steroid hormones play a major role in the integration of reproductive behavior and gonadal function (Fabre-Nys, 1998). In ewes, dopaminemediated D2 receptor (DRD2) signaling in the mediobasal hypothalamus is known to affect female sexual motivation and receptivity (Fabre-Nys et al., 2003). Furthermore, the perception and awareness of male-related cues differ with the stage of estrous cycle, with releases of monoamines (linked to serotonin (HTR2A) and DRD2) and γ-aminobutyric acid (GABA) (linked to GABRA6) in the mediobasal hypothalamus being triggered by such cues only when females are in oestrus (Fabre-Nys et al., 1997). Studies on female rats and hamsters have shown the inhibitory and facilitatory effects of serotonin receptor agonists and antagonists on the hypothalamic regulation of sexual receptivity (Uphouse, 2000; Caldwell and Albers, 2002). This regulation is also mediated by GABAergic neurons interacting with serotonin-containing neurons. Other genes that may be grouped in the Preparation module are those known from studies on other species to play a role in emotional responses and that were found to be associated with the heat score of cows (Kommadath et al., 2011), for example, transthyretin (TTR), myelin-associated oligodendrocyte basic protein (MOBP), Leukotriene A4 hydrolase (LTA4H) and potassium channel, calcium activated intermediate/ small conductance subfamily N alpha, member 2 (KCNN2). TTR has been linked to anxiety (Sousa et al., 2004), MOBP to mood disorders (Sokolov, 2007), LTA4H to depression (Zhao et al., 2009), and KCNN2 to anxiety and stress responses (Mitra et al., 2009).

Permission: The fourth module is permissive actions by hypothalamic neurons for the mating behavior to occur The association between estrous behavior scores and the expression of acetylcholinesterase (ACHE) and several cholinergic receptors (CHRM1, CHRM3 and CHRNA5) can be explained by the effect of the neurotransmitter acetylcholine on arousal, plasticity and reward. The products of the muscarinic cholinergic receptor genes, CHRM1 and CHRM3, are Gq-protein-coupled receptors whose activation releases intracellular Ca2+ via the phospholipase C – inositol 1,4,5-trisphosphate signaling pathway (Billups et al., 2006). The genes for phospholipase C and inositol triphosphate kinase (PLCB2, ITPKA) and several protein kinases were also found associated with estrous behavior scores. These findings can be explained based on the hypothesis put forward by Kow and Pfaff (2004) that the membrane actions of E2 can modulate the genomic actions of E2 and that this transcriptional potentiation is mediated via signaling pathways requiring the activation of certain protein kinases and increased intracellular Ca2+.

Synchronization: The fifth module is to synchronize mating behavior with ovulation. The heat score-associated genes PTGDS, prostaglandin I2 (Prostacyclin) synthase (PTGIS) and prostaglandin F receptor PTGFR (Kommadath

et al., 2011 and 2013) may be grouped in this module. These genes regulate prostaglandin functioning. Prostaglandins are known to be under the influence of E2 (Amateau and McCarthy, 2002) and are capable of directly affecting the neurons that synthesize and secrete gonadotropin-releasing hormone (Jasoni et al., 2005; Clasadonte et al., 2011).

Figure 5 demonstrates estrous behavior-associated miRNA and their genes grouped in the GAPPS modules described for female sexual behavior in dairy cows. miR28, miR155, miR200A, miR326, miR378, miR452, miR483, miR491, miR512-5P and miR525 were associated with regulation of genes involved in GAPPS modules.

Current status and future consideration

Currently, the utilization of artificial insemination has significantly improved the production potential of dairy cows. Unfortunately, for many decades breeding objectives focused solely on milk production which consequently resulted in decline in genetic merit for estrus expression/ fertility traits. The causes responsible for this decline include disproportionate mobilization of body energy reserves, unfavorable metabolic status, delayed resumption of cyclicity, increased incidence of uterine disease, dysfunctional estrus expression and insufficient luteal phase progesterone concentrations (Cummins et al., 2012; LeBlanc 2013). It should be noted that genetic merit for fertility traits had a significant effect on characteristics of the estrous cycle. On a constructive note, it is possible to identify sires that combine good milk production traits with traits for increased estrus expression behavior and good fertility. Sire genetic merit for daughter fertility traits is improving rapidly in the dairy breeds, including the Holstein. With advances in animal breeding, especially genomic technologies, genetic merit for estrus expression and fertility traits can be improved much more quickly than they initially declined (Figure 6).

Conclusion

Current research findings indicate that the decreased expression of estrous behavior seen in high-producing dairy cows is related to decreased blood concentrations of ovarian steroids, delayed resumption of cyclicity, dysfunctional estrus expression and insufficient luteal phase progesterone concentrations, which in turn may partly be explained by an increased rate of hepatic metabolization of ovarian steroids in high-producing dairy cows. A better understanding of the between-cow variation in the ovarian steroid-driven central genomic regulation of estrous behavior in dairy cows may contribute to the development of tools and management strategies to improve estrous behavior detection and therewith reproductive efficiency. This understanding may facilitate the development of a genomic selection strategy for improving the trait of estrous behavior expression, thereby improving reproductive efficiency in high-producing dairy cows.

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Table 1

Estrous behavior-associated genes and processes grouped in the GAPPS modules described for female sexual behavior in dairy cows (Adopted from Mong and Pfaff, 2004)

GAPPS module	Corresponding genes and processes in cows	Action
Growth Increase in the input/output connections for behaviour-directing hypothalamic neurons or synaptic plasticity	Immune-related genes CTLA4; IL1RL1; MARCO Neurotransmitter receptors CHRM1; CHRM3; CHRNA5 Ribosomal genes RPL14; RPL18; RPL24; RPS11; RPS18 Other genes NEFL; NDRG2; THY1	Estradiol-dependent outgrowth of hypothalamic neurons, amygdala and hippocampus, and the anterior pituitary;
Amplification Amplification of estrogen effect by progesterone mediated by progesterone receptor	PGR	Estrous behavior, possibly via ligand-independent pathways;
Preparation Preparation for mating	Female sexual receptivity OXT; AVP; HTR2A; DRD2; GABRA6 Anxiolytic effect OXT; TTR: KCNN2 Altered feeding motivation and mood POMC; MCHR1; MOBP; LTA4H	Sexual receptivity, grooming behavior and partner bonding; Courtship and mating; Modulation of anxiety and satiety, reproductive beavior, motivation and sexual receptivity
Permission Permissive actions by hypothalamic neurons for the mating behaviour to occur	Arousal, activation of protein kinases and release of Ca2+ CHRM1; CHRM3; CHRNA5; PLCB2; ITPKA	Arousal, plasticity and reward, estrous behavior;
Synchronization Synchronize mating behavior with ovulation	Prostaglandin regulators PTGDS; PTGIS; PTGFR	Mating behavior and ovulation

Table 2

Summary of estrus related differences between heifers with good (Fert+) or poor (Fert) breeding values for fertility traits (Adopted from Cummins et al., 2012)

Factors	Estrus/Fertility +	Estrus/Fertility-
Estrus cycle length (days)	21	25.1
Follicular waves/cycle	2.2	22.7
Circulating P4 from day 5 to 13 of estrus cycle (ngmL-1)	5.15 (34%↑)	3.83
Corpus luteum volume	16%↑	-
Silent estrus	2	22
Estrus with no ovulation	0	14
Duration of estrus	7.53	5.86
Peak estrus activity	168	119

Figure 1

Graph illustrating association between first service conception rate (CR) and milk production from 1950 to 2007 in US Holstein dairy cows (Butler, 2003)

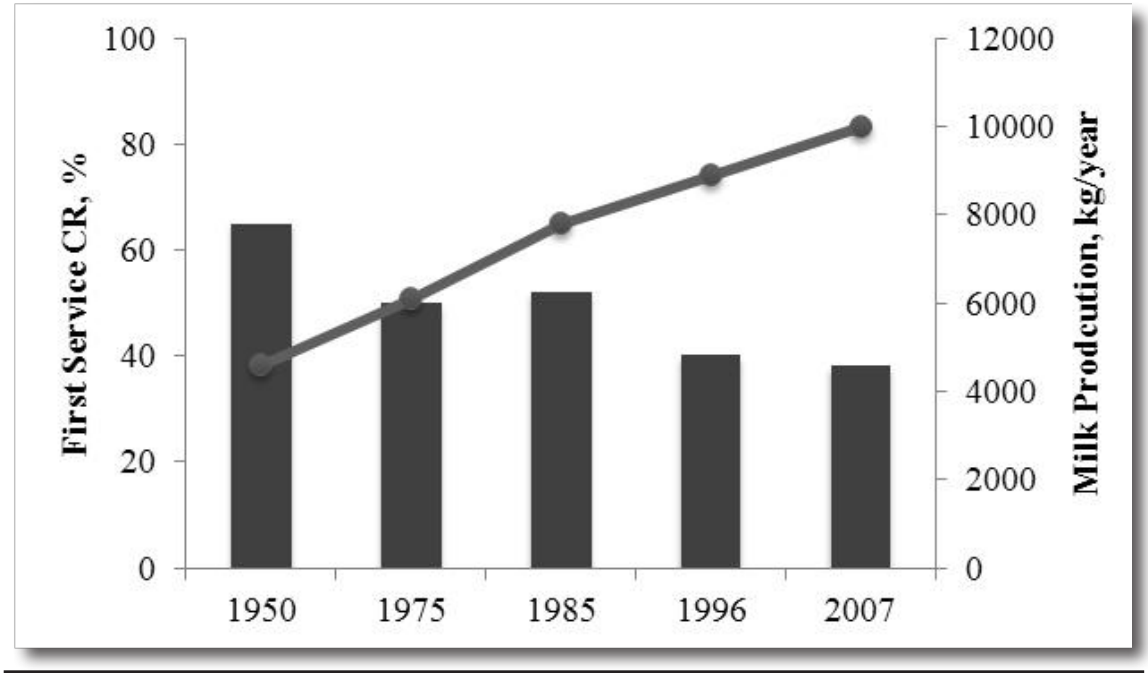


Figure 2

Cows expressing "standing" and "mounting" activity. Standing activity is considered as gold standard for sign of estrus

Figure 3

Estrogen signaling via genomic (Nuclear estrogen receptor [ER]) and non-genomic (Membrane ER) mechanism

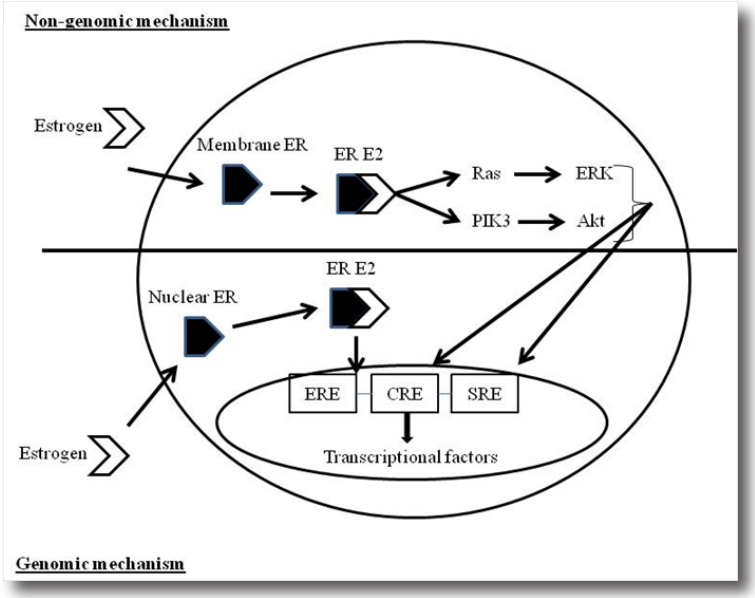


Figure 4

miRNA and associated genes involved in regulation of estrus

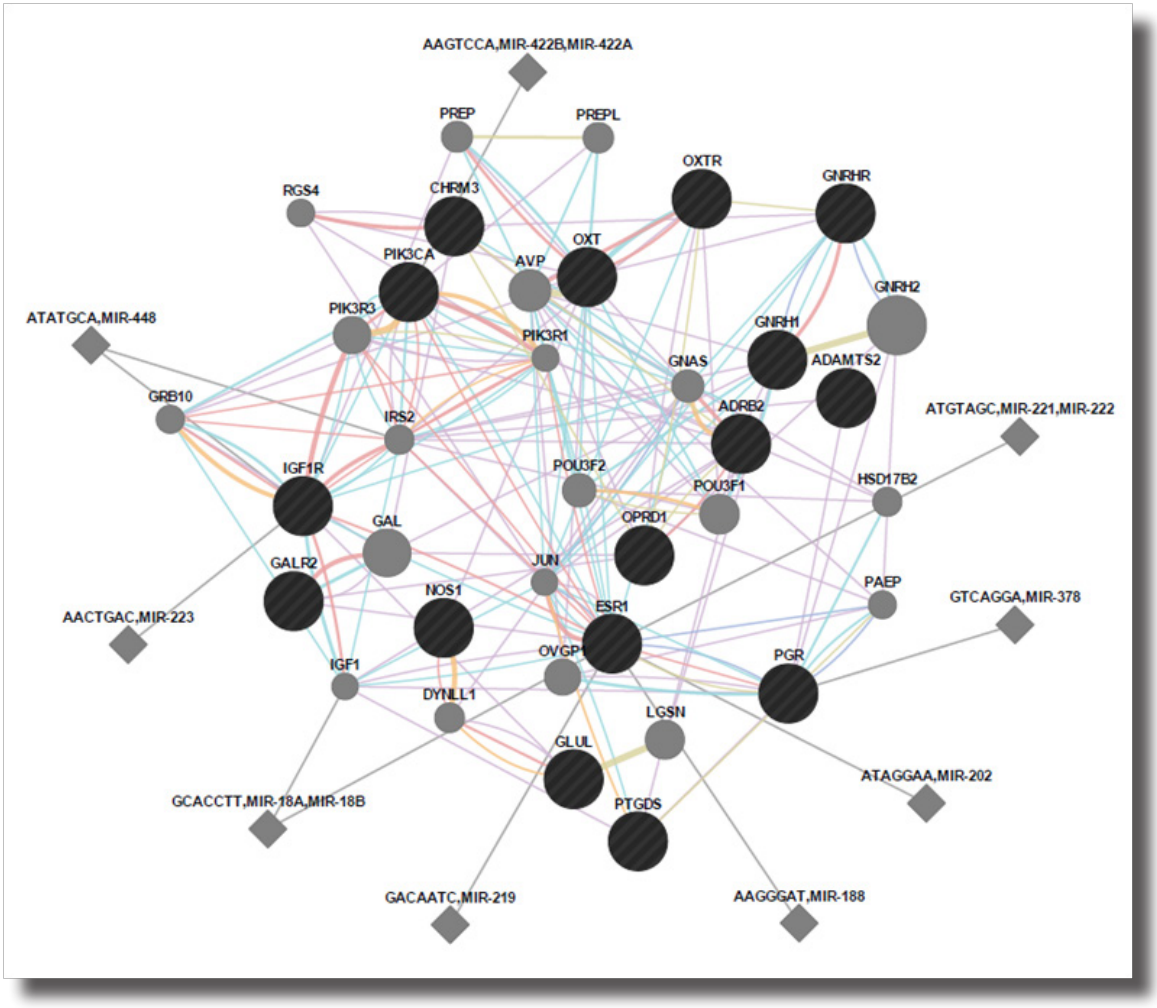


Figure 5

Estrous behavior-associated miRNA and their genes grouped in the GAPPS modules described for female sexual behavior in dairy cows

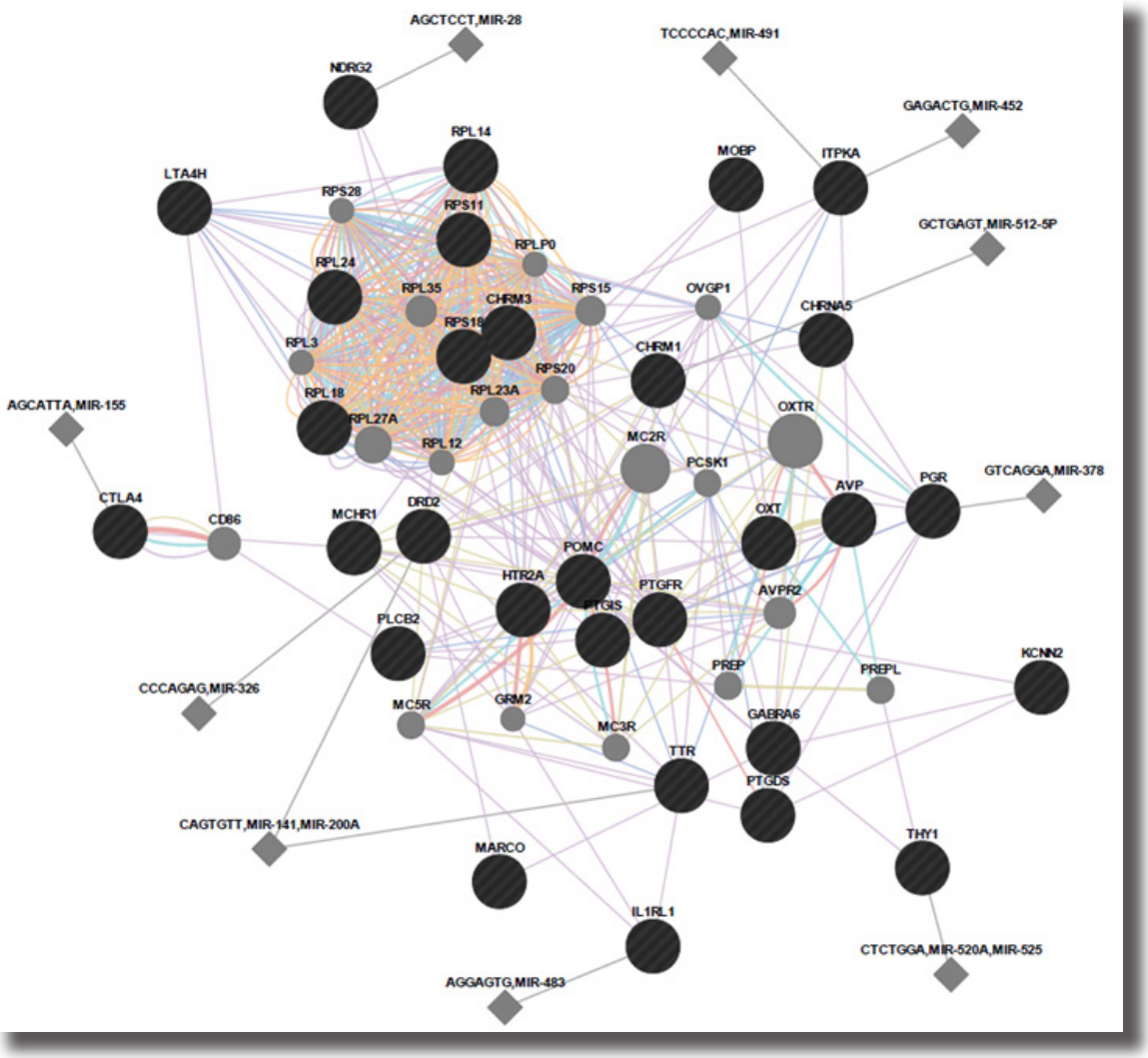
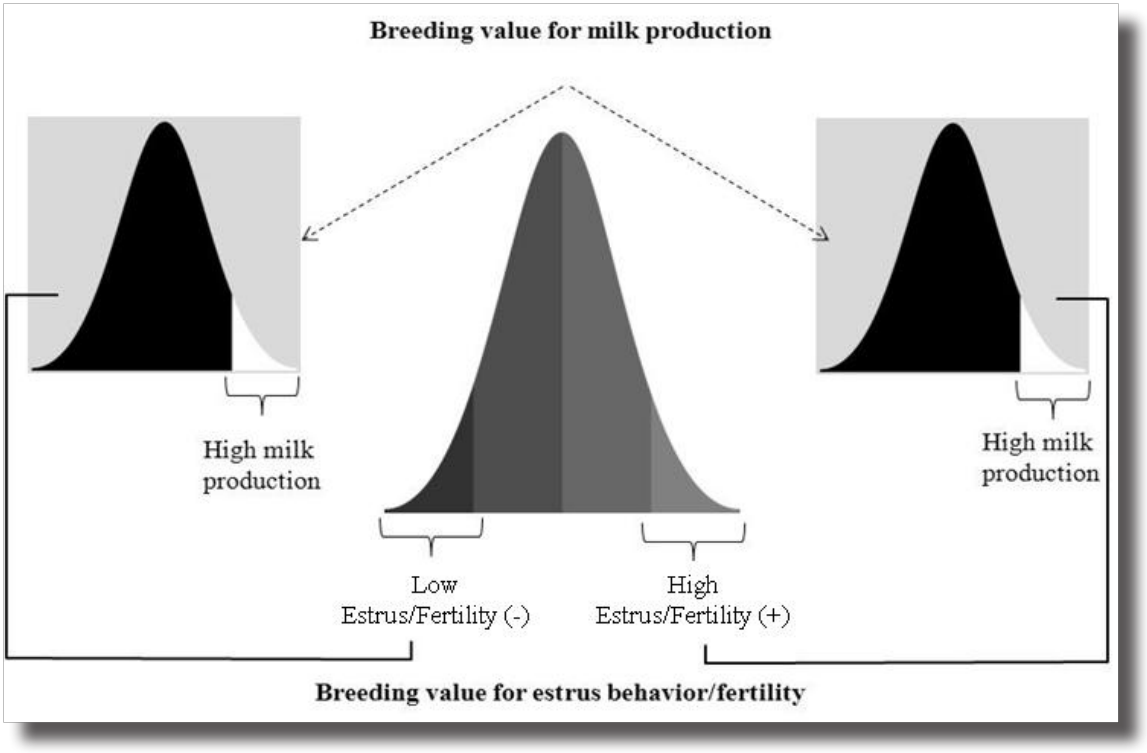


Figure 6

Schematic presentation of the derivation of the animal model (Modified from Butler ST, 2013)



Genetic merit for fertility has pronounced effects on behavioral estrus. Pregnant heifers with good (Estrus/Fertility+) or poor (Estrus/Fertility–) breeding values for fertility traits should be identified within the database. Within these two extremes, animals with breeding values for Estrus/Fertility+ and high milk production should be identified and propagated.