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Estrous behavior in dairy cows: identification of underlying mechanisms and gene functions

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Selection in dairy cattle for a higher milk yield has coincided with declined fertility. One of the factors is reduced expression of estrous behavior. Changes in systems that regulate the estrous behavior could be manifested by altered gene expression. This literature review describes the current knowledge on mechanisms and genes involved in the regulation of estrous behavior. The endocrinological regulation of the estrous cycle in dairy cows is well described. Estradiol (E2) is assumed to be the key regulator that synchronizes endocrine and behavioral events. Other pivotal hormones are, for example, progesterone, gonadotropin releasing hormone and insulin-like growth factor-1. Interactions between the latter and E2 may play a role in the unfavorable effects of milk yield-related metabolic stress on fertility in high milk-producing dairy cows. However, a clear understanding of how endocrine mechanisms are tied to estrous behavior in cows is only starting to emerge. Recent studies on gene expression and signaling pathways in rodents and other animals contribute to our understanding of genes and mechanisms involved in estrous behavior. Studies in rodents, for example, show that estrogen-induced gene expression in specific brain areas such as the hypothalamus play an important role. Through these estrogen-induced gene expressions, E2 alters the functioning of neuronal networks that underlie estrous behavior, by affecting dendritic connections between cells, receptor populations and neurotransmitter releases. To improve the understanding of complex biological networks, like estrus regulation, and to deal with the increasing amount of genomic information that becomes available, mathematical models can be helpful. Systems biology combines physiological and genomic data with mathematical modeling. Possible applications of systems biology approaches in the field of female fertility and estrous behavior are discussed.

Keywords: dairy cow, estrous behavior, physiology, genomics

Implications

In dairy cows, optimal time of artificial insemination is signaled by estrous behavior. Selection for milk yield has coincided with a decline in duration and intensity of estrus, decreasing success of insemination. Hormonal regulation of the estrous cycle in cows is well-described, but a clear understanding of how this is tied to estrous behavior is only starting to emerge. This study reviews mechanisms and genes involved in the regulation of estrous behavior in farm animals and rodents.

Introduction

Dairy cattle selection for higher milk yield has coincided with a decline in fertility (for reviews see Royal *et al.*, 2000; Veerkamp *et al.*, 2003; Pryce *et al.*, 2004). Subfertility in modern dairy cows is a multifactorial problem. It involves factors like genetic improvement for milk yield, nutritional issues, disease, season, climate, housing, management and herd environment (Lucy, 2001; Roche, 2006). The mechanisms by which selection for higher milk yield can result in poorer fertility are not totally elucidated, but one cause is likely to be metabolic stress (Veerkamp *et al.*, 2003). As the reproductive and somatotropic axes interact at several levels in the hypothalamus (Chagas *et al.*, 2007), it is not surprising to find relationships between energy balance and fertility parameters. Subfertility has negative implications for dairy farm profitability, sustainability of animal production and animal welfare, as it takes more time and effort to get cows to be pregnant.

Low estrus detection rate has been identified as an important factor affecting the reproductive efficiency (Lopez *et al.*, 2004). The optimal timing of artificial insemination is signaled by estrous behavior. However, the detection of

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estrus in modern high milk-yield dairy cows is hampered, because the duration and intensity of estrous behavior in these cows is considerably lower than that in dairy cows of a few decades ago (reviewed by Lopez *et al*., 2004). Little is known about heritability and genetic variance of estrous behavior. A recent study reported heritability estimates for estrus duration and intensity to be low (2% to 8%; Lovendahl and Chagunda, 2009). Heritability estimates of fertility traits based on artificial insemination service dates are generally below 5%, while heritability estimates of days from calving to first estrous based on progesterone (P4) profiles or behavior observation are higher (16% to 28%; reviewed by Pryce and Veerkamp, 2001). However, genetic control of estrous behavior as such remains elusive. Changes in the underlying mechanisms that regulate estrous behavior could be manifested by altered gene expression patterns. The study of these gene expression changes could be a means to gain insight into the genomic regulation of estrous behavior in cows. Gene expression studies are useful for discovering the biological principles that underlie polygenic traits (Bertani *et al*., 2004) like estrus. There is limited information on genes that regulate the reproductive behavior in dairy cows, but considerable knowledge is available from other species, especially the rodents. The genes found to be important in rodents and other mammals may also be relevant for reproductive behavior in cows, because of shared neurophysiologic mechanisms.

The aim of this review is to describe the current state of knowledge regarding genomic regulation of estrous behavior in the brain. The first part of this study briefly summarizes the physiological mechanisms involved in estrous behavior of dairy cows, which provides the framework for the main topic: an overview of the current knowledge on relevant genes and their functions in endocrine mechanisms that regulate estrous behavior.

Physiological regulation of the estrous cycle and estrous behavior

General principles of estrus regulation

During pro-estrus, when the corpus luteum (CL) is regressed and the concentration of (P4) is decreased, the dominant follicle, deviated from a cohort of antral follicles, matures under the influence of luteinizing hormone (LH) and follicle stimulating hormone (FSH; Allrich, 1994). FSH plays an important role at the beginning of follicular development, whereas LH is important for follicular growth up to ovulation (Ginther *et al*., 1996). The dominant follicle secretes an increasing amounts of estradiol (E2) during the development to preovulatory size (Allrich, 1994). E2 is involved in important neuroendocrine mechanisms regulating estrus. E2 inhibits gonadotropin releasing hormone (GnRH) secretion from the hypothalamus and LH secretion from the pituitary throughout most of the cycle. However, during pro-estrus, elevated E2 levels increase the secretion of GnRH, which together with direct effects of E2 on the pituitary, triggers the LH surge (Glidewell-Kenney *et al*., 2007), which induces ovulation. Once an oocyte is successfully ovulated, the remains of the follicle form a new P4-producing CL. Progesterone maintains the readiness of the endometrium for receiving the embryo. If conception has failed, the CL regresses, P4 levels decrease and the cycle restarts.

Estrous behavior

The estrous cycle of cows lasts for approximately 21 days. The interval between onset of mounting behavior and ovulation in cows is approximately 27 h (Lopez *et al*., 2002; Roelofs *et al*., 2005b). In modern Holstein cows the duration of estrus, defined as the time between first and last recorded standing event, has been reported to be 7 h (Dransfield *et al*., 1998; Lopez *et al*., 2004). In contrast, Esslemont and Bryant (1976) reported an average duration of estrus of 14.9 h in Friesian cattle in 1976. Table 1 summarizes different behavioral signs of estrus in cows. At the start of estrus, a cow typically sniffs the vulva of other cows and rests her chin on the back of others. Such behavior is followed by mounting of other cows and ultimately the cow displays standing heat (Roelofs *et al*., 2005b). Van Eerdenburg *et al*. (1996) defined a protocol based on these behavioral signs in order to detect whether a cow is in heat. As shown in Table 1, not all cows express all behavior traits. Lyimo *et al*. (2000) and Roelofs *et al*. (2004) showed that the highest behavioral score of cows in estrus, based on the estrous behavior signs given in Table 1, correlates positively with maximum plasma E2 concentrations, but no correlation was found between E2 levels and specific estrous behaviors (Cook *et al*., 1986; Coe and Allrich, 1989). Because the percentage of cows displaying standing heat has declined over the last decades (reviewed by Dobson *et al*., 2008), it is more difficult to detect estrus based on standing heat. Therefore, other methods to detect and quantify estrus have been proposed by Roelofs *et al*. (2005a; pedometers) and Lovendahl and Chagunda (2009; electronic activity tags). Little is known about the underlying mechanisms and the level of genetic control of specific estrous behaviors, but collection of quantifiable data could be helpful in the research of genetic mechanisms (Schutz and Pajor, 2001).

A 'normal' endocrinological cycle is prerequisite for estrus and estrous behavior. However, ovulation is not necessarily

Table 1 *Behavioral signs of estrus in cows**

Estrous signs	Percentage of estruses in which the behavior is displayed
Flehmen	44
Sniffing vulva of another cow	100
Mounted but not standing	56
Resting with chin on back of another cow	100
(Attempt to) mount another cow	90
(Attempt to) mount head side of another cow	22
Standing heat	56

*Adapted from Van Eerdenburg *et al*. (2002) and Roelofs *et al*. (2005b).

accompanied by estrous behavior ('silent estrus' (Allrich, 1994), indicating that physiological events and behavior are in part based on different mechanisms. In dairy cows, the first postpartum ovulation occurs often without clear signs of estrous behavior (Kyle *et al*., 1992). This 'silent estrus' is thought to be a result of high E2 concentrations from fetal origin at the end of gestation, which induces 'refractoriness' in the hypothalamus to E2 at the first postpartum ovulation. The CL produced after the first ovulation provides the P4 that removes this refractory state and facilitates the behavioral expression of the subsequent estrus (Allrich, 1994).

Endocrine regulation of estrous behavior: the central role of E2
E2 plays a key role in the regulation of endocrine and behavioral events associated with the estrous cycle. In many experiments that are performed to study the female reproduction, estrus is artificially induced by administering E2 (e.g. Fabre-Nys *et al*., 1993). E2 plays a central role in triggering the gonadotropin surge and ovulation as well as in facilitating 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 (Smith and Jennes, 2001) and P4 (Richter *et al*., 2005; Zalányi, 2001). E2 stimulates 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 (reviewed by Reinecke and Deuflhard, 2007), which results in the LH surge. The shift from inhibition to stimulation may be dependent on the site of action of E2, that is, a switch from membrane signaling to genomic signaling (Arreguin-Arevalo and Nett, 2006). The LH surge is driven by an increased pituitary responsiveness to GnRH, which is determined by the amount of GnRH receptors (GnRH-Rs) expressed on gonadotropes. Pulsatile GnRH release, facilitated by high E2 concentrations during the preovulatory period, elevates *GnRH-R* gene expression, whereas high P4 concentrations in the luteal phase inhibits the *GnRH-R* gene expression (reviewed by Weiss *et al*., 2006).

E2 plays a pivotal role in the induction of estrous behavior (Pfaff, 2005). It has a self-amplifying effect as it stimulates the expression of estrogen receptors (ER**s**) in the brain, which is thoroughly investigated in rodents (Pfaff *et al*., 2008). The duration of estrous behavior in sheep 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, for example, 0.4 μg/kg in sheep and 10 μg/kg in rats (Fabre-Nys and Gelez, 2007). In sheep, Saïd *et al*. (2007) demonstrated that estrous behavior required lower E2 concentrations than required for the LH surge and that estrous behavior can be induced independently of the LH surge. Estrous behavior and the LH surge cannot only be separated by experimental reduction of E2 levels, but also by stress (Dobson *et al*., 2008). Lameness, an example of a stress inducing condition, was found to reduce behavior score (based on signs given in Table 1) of cows in estrus (Walker *et al*., 2008) and to inhibit LH surge and ovulation (Dobson *et al*., 2008) whereas incidence of estrus was not reduced (Walker *et al*., 2008), which could result in lower pregnancy rates. These observations suggest that stress, caused by lameness, reduces P4 exposure before estrus (Walker *et al*., 2008) 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).

Metabolic disturbances
High milk production affects the energy metabolism, which can disturb the endocrine signaling (Roche, 2006). Altered energy metabolism in high milk yielding cows can cause decreased levels of E2 and inhibit estrous behavior (Lopez *et al*., 2004). Cows selected for high milk yield are genetically induced to a more negative energy balance (Veerkamp *et al*., 2003) as they spend a relatively large proportion of the available nutrients on milk production, which can cause fertility problems during a period of negative energy balance (Chagas *et al*., 2007). One possible route by which metabolic stress can inhibit estrous behavior is via insulin-like growth factor-1 (IGF-1). IGF-1 production is inhibited during negative energy balance. IGF-1 receptor signaling in the brain (Velazquez *et al*., 2008) is needed for the positive effect of E2 on the release of LH and for normal E2 priming of estrous behavior (Etgen *et al*., 2006; Mendez *et al*., 2006). Furthermore, concentrations of other metabolic factors that are known to affect dairy cow fertility, for example, insulin, leptin and growth hormone, interact with IGF-1 levels (Diskin *et al*., 2003; Chagas *et al*., 2007).

Changes in reproductive physiology that are associated with high milk production may in part be explained by elevated P4 and E2 clearance rates, as described in the physiological model of Wiltbank *et al*. (2006). In this model, clearance rates of hormones by the liver of a lactating cow are increased as a result of elevated feed intake, leading to an increased liver blood flow and metabolic activity. With a similar level of hormone production, circulating hormone levels would thus be lower. The model also provides an explanation for decreased duration of estrus: elevated E2

metabolism means a more rapid decrease in circulating E2 after the LH surge. Combining the facts that E2 is an important regulator of estrous behavior in cows (Lyimo *et al*., 2000) and that increased level of milk production is associated with decreased E2 concentrations (Lopez *et al*., 2004), smaller follicular size (Diskin *et al*., 2003) and shorter duration of estrus (Wiltbank *et al*., 2006), it seems reasonable to conclude that lower E2 levels are (partly) responsible for the poor behavioral expression of estrus in modern dairy cows (Chagas *et al*., 2007).

Genomic regulation of estrous behavior: central mechanisms in the brain

General endocrinological mechanisms of the estrous cycle have been amply studied, but the understanding of the regulation of estrous behavior is only starting to emerge. Genomic approaches are often used to study physiological mechanisms. Differential expression of genes between different time points in the reproductive cycle or between animals with differences in fertility traits could indicate which genes and pathways are relevant for the regulation of estrus. This section reviews recent insights from several research areas regarding genomic regulation of estrous behavior in rodents and other mammalian species. It highlights main mechanisms, rather than dealing with all that are known to play a role, and illustrates the complex interactions between genes, hormones and their receptors that together form the signaling pathways that coordinate the synchronization of mating and ovulation.

Estrogen signaling in the brain

Brain areas that are known to be involved in the regulation of female sexual behavior include the arcuate nucleus (ARC), ventromedial nucleus (VMN) and preoptic area (POA) of the hypothalamus (reviewed by Molenda-Figueira *et al*., 2006). In addition to these areas of the hypothalamus, the hippocampus and amygdala are known to regulate the behavioral aspects of estrus. The amygdala (Zhou *et al*., 2005) and hippocampus (Frye and Rhodes, 2008) are involved in the reduction of anxiety and aggression, and in this way can facilitate sexual behaviors that result from generalized arousal of the brain. E2 and other hormones cause up- or downregulation in these brain areas of a number of genes that are believed to be involved in estrous behavior (Table 2). E2 increases the sensitivity of neurons for itself by inducing *ER* gene expression (Walf and Frye, 2006 and 2008). The E2-receptor complex acts as transcription factor that regulates the expression of a large number of genes (Molenda-Figueira *et al*., 2006). Apart from genomic (classical ER) signaling, the estrogenic control of estrous behavior also involves membrane signaling mechanisms via secondary messengers like phosphoinositide 3 kinase, cAMP response element binding proteins and extracellular signal regulated kinases

Table 2 *Overview of above-mentioned genes involved in the regulation of estrus*

Tissue	Gene	Expression induced by	Effect	Reference
Hypothalamus	ERα, ERβ	E2	Induces expression of other genes, facilitating estrous behavior	Pfaff *et al*. (2008)
	rRNA and growth	ERα	Facilitates estrous behavior	Pfaff *et al*. (2008)
	nNitric oxide synthase	ERα	Mediates neuro-transmission	Pfaff *et al*. (2008), Mani *et al*. (1994), Sica *et al*. (2009)
	Adrenergic and muscarinic receptors	ERα	Promotes neuronal excitability by modulating potassium channels	Lee and Pfaff (2008), Pfaff *et al*. (2008)
	Enkephalin and opioid receptors	ERα	Analgesia	Pfaff (2005), Pfaff *et al*. (2008)
	Oxytocin and its receptor	ERα, ERβ	Anxiety reduction	Pfaff (2005), Pfaff *et al*. (2008)
	Progesterone receptor	ERα, ERβ	Stimulatory effect on lordosis	Pfaff *et al*. (2008)
	GnRH, GnRH-R	ERα	Synchronizes estrous behavior with LH peak	Pfaff *et al*. (2008), Pfaff (2005)
	Prostaglandin-D synthase	Downregulated by ERα	Anxiety reduction	Pfaff *et al*. (2008), Mong *et al*. (2003d)
	Glutamine synthetase	E2	Neuro-transmission	Blutstein *et al*. (2006)
	Genes involved in PI3K pathway	E2, IGF-1	Involved in E2 signaling	Etgen and Acosta-Martinez (2003), Malyala *et al*. (2004)
	IGF-1 receptor	E2, IGF-1	Growth of dendrites and synapses	Etgen *et al*. (2006), Mendez *et al*. (2006)
Amygdala	Oxytocin and its receptor	ERα, ERβ	Social recognition	Pfaff (2005)
Hippocampus	Glutamine synthetase	E2	Neurotransmission	Blutstein *et al*. (2006)
Pituitary	GnRH-R	E2, GnRH	Pituitary sensitivity to GnRH	Hapgood *et al*. (2005), Weiss *et al*. (2006)
	Progesterone receptor	E2	LH release	Attardi *et al*. (2007)

ERα = estrogen receptor-α; ERβ = estrogen receptor-β; E2 = estradiol; P4 = progesterone; GnRH = gonadotropin releasing hormone; GnRH-R = gonadotropin releasing hormone receptor; PI3K = phosphoinositide 3 kinase; IGF-1 = insulin-like growth factor-1; LH = luteinizing hormone.

(Mendez *et al*., 2006; Kelly and Rønnekleiv, 2009; Micevych and Dominguez, 2009).

Arousal and lordosis behavior in rodents

Rodents are often used as a model to study the regulation of fertility in mammals, for example, using genomic approaches (e.g. Laissue *et al*., 2009). The gene expression studies of Pfaff and coworkers revealed several mechanisms that are involved in arousal (Frohlich *et al*., 1999) and, more specific, lordosis in rodents (Kow and Pfaff, 1998). Arousal, a general activation of brain and behavior, precedes the lordosis response and results from signaling by neurotransmitters like norepinephrine (Lee and Pfaff, 2008). The expression in the brain of estrogen receptor-α (ERα) and its downstream effects are essential for arousal, as knockout of ERα reduced arousal responses in mice (Garey *et al*., 2003; Mong *et al*., 2003a). E2-induced down-regulation of prostaglandin-D synthase in the POA increases arousal response (Mong *et al*., 2003b) and prostaglandin-D synthase downregulation is associated also with lordosis (Pfaff *et al*., 2008). The initial step in the induction of lordosis is the E2 controlled alteration of neuronal activity in the 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 (Lee *et al*., 2008) 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 lordosis behavior (reviewed by Lee and Pfaff, 2008). Another example is the E2-induced expression of glial specific genes, including glutamine synthetase, in the ARC and VMN nuclei, and in the amygdala and hippocampus, thus facilitating the glutamatergic neurotransmission important for estrous behavior (Blutstein *et al*., 2006). At least nine genes, expressed in the rodent hypothalamus, are known to be turned on following the binding of estrogen to its receptor. In the VMN, binding of E2 to ERα activates the expression of genes for rRNA and growth, nNitric oxide synthase, adrenergic and muscarinic receptors, enkephalin and opioid receptors, P4 receptor, and oxytocin and oxytocin receptor (Table 2). In addition, binding of E2 to ERβ activates genes for P4 receptor, and oxytocin and oxytocin receptor. In the POA, E2 binding to ERα upregulates *GnRH* and *GnRH-R* genes and downregulates prostaglandin-D synthase (summarized by Pfaff *et al*., 2008). Together, the products of these genes play a role in the induction of the behavioral expression of estrus. A recent study of Sica *et al*. (2009) in female mice showed changes in nNitric oxide synthase expression in the hypothalamus during the estrous cycle. Increased numbers of nNitric oxide synthase immunoreactive neurons were found in the ARC during proestrus and in the POA during estrus. As these regions show large numbers of ERα, this study supports the conclusion of Pfaff and coworkers that E2 modulates expression of nNitric oxide synthase, which stimulates estrous behavior via activation of the nitric oxide signaling pathway (Sica *et al*., 2009). The estrogen-induced regulation of lordosis and synchronized ovulation in rodents can be described in five modules (Pfaff, 2005). (i) Preceding estrus, E2 induces expression of genes involved in growth of dendrites and synapses of VMN neurons that are involved in facilitating sexual behavior. (ii) P4 administration after estrogen priming amplifies the effect of estrogen on reproductive behavior via upregulation of several transcripts. (iii) The presence of estrogens induces expression of several genes (examples are mentioned above) involved in behaviors that prepare the animal for mating. These genes establish analgesia, social recognition and reduction of anxiety and aggression. (iv) E2 induced upregulation of neurotransmitter receptors in VMN neurons primes the neural circuit that triggers the lordosis behavior. (v) E2 elevates GnRH, which stimulates the ovulatory gonadotropin release and facilitates estrous behavior. As E2, through its effects on GnRH and LH, also regulates the LH surge and ovulation, E2 indirectly synchronizes mating and ovulation. The lordosis reflex has been used as a behavioral model to study the functioning of serotonin (Uphouse, 2000; Uphouse *et al*., 2007) and E2 signaling (Micevych and Dominguez, 2009; Micevych *et al*., 2009). Although these studies do not aim directly to unravel the regulation of estrous behavior, they support the findings of Pfaff and coworkers that E2 induces estrous behavior via *ER* gene expression and membrane signaling.

Near to no research has been carried out on E2-induced gene expression related to induction of estrous behavior in cattle, but there are reasons to assume that the mechanisms are similar. Estrous behavior in ruminants, like cows and sheep, is controlled by E2 levels (Lyimo *et al*., 2000; Saïd *et al*., 2007). In rodents, E2 reduces anxiety and therewith stimulates locomotion and exploratory behavior (Mong and Pfaff, 2003c), and a similar E2-induced increase in activity is seen in cows (Roelofs *et al*., 2005a). Another parallel that can be drawn between rodents and ruminants is that the brain areas shown to be highly involved in regulation of estrous behavior in rodents (ARC, VMN and POA) are also the regions with high concentrations of ER during estrus in ewes (Lehman *et al*., 1993; Stormshak and Bishop, 2008). Most gene expression studies dealing with bovine fertility are focused on follicle development and changes in ovarian tissue (Zielak *et al*., 2007; Mihm *et al*., 2006). Beerda *et al*. (2008) compared gene expression profiles in brain samples of Holstein Friesian heifers in (pro-)estrus to those in heifers in luteal phase. Quantitative scores for estrous behavior are linked to gene expressions in the pituitary, hypothalamus, hippocampus, amygdala and ventral tegmental area (VTA). The first analyses of the VTA indicated that cyclical changes in the expression of genes regulating cell morphology and adhesion were linked to the appropriate expression of estrous behavior in dairy cows.

Understanding the complex regulation of estrous behavior

A considerable part of the reported study in this review revolves around control of ovarian E2 production and the LH

surge rather than regulation of estrous behavior, because relatively little research has been carried out on the control of estrous behavior as such. Gene expression profiling is a powerful tool for the identification of genes and mechanisms underlying estrous behavior in dairy cows. The sequencing of the genome of diverse animal species provided a huge amount of data and the biological interpretation of these data has just begun. Large datasets are being generated by functional genomics approaches, like measuring differential expression of genes related to fertility. Various bioinformatics and other post-analysis approaches are being used in order to integrate these data in physiological concepts. Reproductive behavior is a result of numerous gene products, cooperating in pathways that finally induce or facilitate a behavioral response. The number of factors involved in the regulation of estrus is overwhelming and mirrors complex networks. To improve the understanding of bovine reproductive behavior, it might be supportive to integrate the involved physiological and genomic components to describe the various mechanisms that are involved in the interplay of relevant brain areas like the hypothalamus, and the pituitary and ovaries.

To understand complex biological networks like the regulation of estrus, mathematical models and simulation studies can be helpful (Potter and Tobin, 2007). Mathematical models have been developed, for example, for follicle development (Clément *et al*., 2001; Soboleva *et al*., 2004), gonadotropin release (Blum *et al*., 2000; Heinze *et al*., 1998; Washington *et al*., 2004), and estrogen signaling (Vasudevan and Pfaff, 2008; Frohlich *et al*., 2002). The coupling of physiological and genomic data with the help of modeling (e.g. gene network models or mechanistic mathematical models) aims to improve insight in the biological system as a whole (Burbeck and Jordan, 2006), and this approach is often referred to as systems biology. An interesting example of a systems biology approach in the field of female reproduction is the model for the human menstrual cycle developed by Reinecke and Deuflhard (2007), which integrates the major tissues and hormones involved, and is able to simulate the dynamics of follicular development and the associated cyclic hormone level changes. It is expected that a systems biology approach improve the understanding of physiological consequences of alterations in gene expression patterns, for example, the possible implications for expression of estrous behavior. Systems biology approaches, including the use of network models and mechanistic mathematical models, are likely to play a role in further increasing our understanding of the complex interplay of factors involved in the reproductive cycle and the regulation of estrous behavior.

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