

Central genomic regulation of the expression of oestrous behaviour in dairy cows: a review

H. Woelders^{1a}, T. van der Lende^{2a}, A. Kommadath^{1b}, M. F. W. te Pas¹, M. A. Smits^{1†} and L. M. T. E. Kaal¹

¹Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 65, 8200 AB Lelystad, The Netherlands; ²ProPhys Animal Science Consultancy, Klaproosdreef 21, 5288 JP Swifterbant, The Netherlands

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The expression of oestrous behaviour in Holstein Friesian dairy cows has progressively decreased over the past 50 years. Reduced oestrus expression is one of the factors contributing to the current suboptimal reproductive efficiency in dairy farming. Variation between and within cows in the expression of oestrous behaviour is associated with variation in peripheral blood oestradiol concentrations during oestrus. In addition, there is evidence for a priming role of progesterone for the full display of oestrous behaviour. A higher rate of metabolic clearance of ovarian steroids could be one of the factors leading to lower peripheral blood concentrations of oestradiol and progesterone in high-producing dairy cows. Oestradiol acts on the brain by genomic, non-genomic and growth factor-dependent mechanisms. A firm base of understanding of the ovarian steroid-driven central genomic regulation of female sexual behaviour has been obtained from studies on rodents. These studies have resulted in the definition of five modules of oestradiol-activated genes in the brain, referred to as the GAPPS modules. In a recent series of studies, gene expression in the anterior pituitary and four brain areas (amygdala, hippocampus, dorsal hypothalamus and ventral hypothalamus) in oestrous and luteal phase cows, respectively, has been measured, and the relation with oestrous behaviour of these cows was analysed. These studies identified a number of genes of which the expression was associated with the intensity of oestrous behaviour. These genes could be grouped according to the GAPPS modules, suggesting close similarity of the regulation of oestrous behaviour in cows and female sexual behaviour in rodents. A better understanding of the central genomic regulation of the expression of oestrous behaviour in dairy cows may in due time contribute to improved (genomic) selection strategies for appropriate oestrus expression in high-producing dairy cows.

Keywords: sexual behaviour, dairy cow, oestrus, gene expression, brain

Implications

Reduced oestrus expression is one of the factors contributing to the current suboptimal reproductive efficiency in dairy cows. A recent series of publications that correlated the level of oestrous behaviour expression to gene expression levels in the cow brain and anterior pituitary are reviewed here and their findings placed in perspective of what is known about sexual behaviour from studies on other species. The improved understanding of the central genomic regulation of oestrous behaviour expression may in due time contribute to improved (genomic) selection strategies for appropriate oestrus expression in high-producing dairy cows.

Introduction

Maintaining good fertility and thereby optimum reproductive performance in dairy cows is of great economic importance for the dairy industry. Artificial insemination of cows is widely practiced in modern dairy farming. To ensure timely insemination and thereby maximize conception rates in the herd and profitability of the farm, it is important that the farmer does not miss the relatively short period in the oestrous cycle of the cow, called oestrus, when it is fertile and sexually receptive. In the absence of bulls in the farm, the farmer himself detects cows in oestrus by depending on observations of a number of physical and behavioural signs, collectively known as oestrous behaviour, that a cow exhibits only during oestrus or at a higher frequency during oestrus compared with the rest of its oestrous cycle. The physical signs include mucous discharges from the vulva, swelling of the vulva and reddening of the vaginal mucosa.

^a These authors contributed equally to this paper.

^b Present address: Alberta Ingenuity Centre for Livestock Genomics Technology, University of Alberta, Edmonton, Alberta, Canada.

[†] E-mail: mari.smits@wur.nl

The behavioural signs, studied in detail by Hurnik *et al.* (1975) and Esslemont *et al.* (1980), include restlessness and mounting activities as well as increased levels of nudging, chin-resting, sniffing and licking of the genital area of other cows, and flehmen. Mounting activities include both mounting of other cows and being mounted by other cows. Only a cow in oestrus, when mounted, will show an immobilization reflex referred to as standing oestrus or standing heat. This is the only behavioural sign for true oestrus and is considered the primary sign of oestrus. All other behavioural signs of oestrus as well as the physical signs are useful to identify cows that are suspected to be in oestrus but are not conclusive. Hence, they are collectively referred to as secondary signs of oestrus.

Over the past 50 years, the percentage of Holstein Friesian dairy cows exhibiting standing oestrus (true oestrus) has declined from 80% to 50% and the average duration of true oestrus has progressively decreased from 15 to 18 h to <8 h (Dobson *et al.*, 2008; Reames *et al.*, 2011). In addition, the intensity with which secondary behavioural signs of oestrus are displayed have also steadily declined, even to the extent that some cows show no oestrus signs at all ('silent' oestrus). The reduced duration and intensity, or even the absence, of oestrous behaviour makes it difficult for dairy farmers to detect cows in oestrus, that is, oestrus may go undetected, or weak oestrous signs in luteal cows may be misinterpreted as 'oestrus'. As timely and accurate oestrus detection is essential for successful artificial insemination, the decreased oestrus detection rates contribute significantly to the multi-factorial problem of suboptimal reproductive efficiency in modern dairy farming (Walsh *et al.*, 2011). Using cow information, reproductive dynamics and economics representative for the dairy situation in the Netherlands, Inchausti *et al.* (2010) predicted that an improvement in the oestrus detection rate from 0.30 to 0.50 and from 0.50 to 0.70 will reduce the net economic loss by €53.29 and €11.20 per cow per year, respectively. Various oestrus detection methods aimed at improving detection rate have been developed, evaluated and compared (Diskin and Sreenan, 2000; Firk *et al.*, 2002; Roelofs *et al.*, 2010; Holman *et al.*, 2011). Despite this, modern dairy farming would substantially benefit from an improved oestrus detectability via an increased expression of oestrous behaviour in cows, that is, if cows would generally show the various signs of oestrus during a longer period of time and at a higher intensity and if more cows would show standing oestrus.

A number of studies have reported considerable variations in the durations of true oestrus among cows observed in herds (see review by Dobson *et al.*, 2008). These studies do not directly discriminate between within-cow and between-cow variation in oestrus duration, as cows were generally observed for only one or two consecutive cycles. However, the fact that duration of oestrus is often seen to be associated with traits such as parity (Roelofs *et al.*, 2005) or milk yield (Lopez *et al.*, 2004) suggests that there is a cow component in the variation in oestrous duration. Lopez *et al.* (2004) reported a negative correlation between the duration

of true oestrus (recorded by a radio telemetry system) and the average milk production during the 10 days preceding the day of oestrus ($r = -0.51$; $P < 0.0001$). At the same time, within a given range of milk production levels, there were still considerable variations in the durations of true oestrus among cows. For cows with an average milk yield of 25 to 30 kg/day, the duration of true oestrus varied from 6 to 27 h, whereas for cows with an average milk yield of 45 to 50 kg/day the duration of true oestrus varied from 0 to 18 h. In addition to the duration of true oestrus, the intensity with which typical behavioural oestrous signs (including standing-to-be-mounted) are displayed is also highly variable between cows (Dransfield *et al.*, 1998; Yoshida and Nakao, 2005). The intensity of oestrus is often expressed as a cumulative score for weighted behavioural signs. The scoring method proposed by Van Eerdenburg *et al.* (1996), shown in Table 1, has been frequently used. The intensity of oestrous behaviour expression is not only important for successful artificial insemination but also for high conception and calving rates once inseminated. Several studies have shown that cows with a more intense oestrous behaviour (including the standing response) have higher conception and calving rates (Dransfield *et al.*, 1998; Garcia *et al.*, 2011; Gilmore *et al.*, 2011). Oestrous behaviour is thus a key fertility trait in cows.

Understanding the background of individual differences in duration, and especially intensity of oestrous behaviour, may lead to opportunities to improve oestrus expression and reduce the fraction of cows in which intensity and duration of oestrus behaviour are too low for adequate and efficient heat detection. Here we focus on selected aspects of the physiological, neurophysiological and central genomic background of between- and within-cow variation in the expression of oestrous behaviour. We review recent information from literature concerning the interaction between milk production level, blood concentrations of ovarian steroids (oestradiol and progesterone) and the expression of oestrous behaviour. We also review the current knowledge on ovarian steroid-driven

Table 1 The scoring method of Van Eerdenburg *et al.* (1996) for the quantification of the intensity of expression of oestrous behaviour in dairy cows

Oestrous signs	Points ¹
Mucous vaginal discharge	3
Cajoling (flehmen)	3
Restlessness	5
Sniffing the vagina of another cow	10
Mounted but not standing	10
Chin resting	15
Mounting (or attempt) other cows	35
Mounting head side of other cow	45
Standing heat	100

¹Each time a sign is observed during an observation period of 30 minutes, the assigned number of points is recorded. The sum of points per observation period is a measure for the intensity of expression of oestrous signs during the observation period. If the sum of the points exceeds 50 during two consecutive observation periods, a cow is considered to be in oestrus.

mechanisms in the central nervous system that relate to the expression of female sexual behaviour. Although much of this knowledge is based on research on species other than cattle, this review includes a series of recent studies in cattle and discusses how the results of these studies fit in, and connect with, the existing knowledge from studies on other species, especially rodents. Throughout this review, emphasis will be on endocrine, neuroendocrine and functional genomic aspects of female sexual (oestrous) behaviour. Thus, the aim of the current review is to integrate knowledge on endocrine regulation of oestrous behaviour of modern high-yielding dairy cows with knowledge on genomic regulation of oestrous behaviour in various animal species, including cattle. With this review, we hope to stimulate integrated physiological and molecular genetic research on the regulation of oestrous behaviour expression in dairy cows. Increased knowledge in this field may contribute to the development of tools for detecting oestrous behaviour or to improvement of oestrus expression through changes in animal management, or even to the development of breeding strategies for genetic improvement in the trait.

Ovarian steroid hormones and oestrous behaviour expression in dairy cows

Sexual arousal is dependent on neural (sensory and cognitive) factors, hormonal factors and genetic factors. It activates cognitive and physiological processes that can eventually lead to sexual behaviour (Schober and Pfaff, 2007). Regarding hormonal factors, Schober and Pfaff (2007) stated that sexual arousal in female animals 'is slavishly dependent on oestrogen and progesterone'. During the oestrous cycle, the sequential actions of the ovarian steroid hormones progesterone and oestradiol in the central nervous system cause cyclical fluctuations in the degree of sexual arousal and facilitate expression of oestrous behaviour just before ovulation. The actions of ovarian steroids on the chain of events constituting female sexual behaviour have been well studied in rodents (reviewed by Blaustein, 2008 and Pfaff *et al.*, 2011).

It has been suggested that the effect of oestradiol on oestrous behaviour in dairy cows is 'all or none': oestrous behaviour is induced as soon as a threshold of oestradiol

concentration is reached and additional oestradiol above this threshold will not further enhance the duration and intensity of behavioural oestrus expression (Allrich, 1994). Although this might have been the case before intense selection for milk yield commenced, the situation seems different now. Several studies with modern high producing dairy cows indicate a relatively strong positive relationship between the oestradiol blood concentration during the late follicular phase and the duration and intensity of behavioural oestrus. For instance, Lyimo *et al.* (2000) (Table 2) found a significant correlation ($r = 0.56$; $P = 0.023$) between the maximum oestradiol concentration during the oestrous phase of the cycle and the total oestrus score as measured using the scoring method of Van Eerdenburg *et al.* (1996). In the same study, the correlation of maximum oestradiol level with the length of the oestrous period was not significant ($r = 0.45$; $P = 0.081$). However, after correction for the variation between cows in progesterone concentration during dioestrus, both correlations were significant ($r = 0.66$, $P = 0.006$ for oestrus score and $r = 0.60$, $P = 0.014$ for duration of oestrus). In agreement with this, Lopez *et al.* (2004) (Table 2) reported a correlation coefficient of 0.57 ($P < 0.0001$) between the oestradiol concentration on the day of oestrus and the duration of oestrus as recorded by a radio telemetry system. In addition, these authors found that the oestradiol concentration was negatively correlated with the average milk production of the 10 days before the day of oestrus ($r = -0.57$; $P < 0.0001$). In low-producing cows, the oestradiol concentrations 1 day before oestrus and on the day of oestrus were 9.6 ± 0.5 and 8.6 ± 0.5 pg/ml, respectively, whereas in high producers these were lower at 7.7 ± 0.7 and 6.8 ± 0.5 pg/ml, respectively (low- and high-producing cows were defined based on whether the average milk production was below or above 39.1 kg/day in the 10 days before the day of oestrus).

Regarding the role of progesterone in the expression of behavioural oestrus in ruminants, some conflicting results have been reported, especially when considering the experiments on ovariectomized ruminants treated with progesterone and/or oestrogens. Nevertheless, there is much evidence from studies with intact ruminants that suggests an important facilitatory role of progesterone priming. This role includes an increase in the oestradiol responsiveness of

Table 2 Blood oestradiol concentration (mean and range) and correlations of oestradiol concentration with oestrous score, oestrus length and milk yield, respectively (*r*-values, with *P*-values between brackets)

	Oestradiol (pg/ml) ¹		<i>r</i> (<i>P</i> -value)		
	Mean	Range	Oestrus score ²	Oestrus length	Milk yield ³
Lyimo <i>et al.</i> (2000)	7.76	0.75 to 13.86	0.56 (0.023)	0.45 (0.081)	
Lyimo, corrected ⁴			0.66 (0.006)	0.60 (0.014)	
Lopez <i>et al.</i> (2004)	7.8	2.1 to 14.6		0.57 (<0.0001)	-0.57 (<0.0001)

¹Maximum level in oestrous phase in Lyimo; level on day of oestrus in Lopez.

²Using the scoring method of Van Eendenburg *et al.* (1996).

³Average milk production for the 10 days before the day of oestrus.

⁴After correction for the variation between cows in progesterone concentration during dioestrus.

specific areas of the central nervous system (especially the hypothalamus) involved in the expression of behavioural oestrus. Several experiments on ovariectomized ewes treated with progesterone and/or oestradiol showed that progesterone priming is needed to induce complete oestrous behaviour after oestradiol injections (reviewed by Fabre-Nys and Gelez, 2007). Similar results have been found in ovariectomized goats, although the effectiveness of progesterone priming was affected by seasonal breeding status and the time interval between the sequential progesterone and oestradiol treatments (reviewed by Katz, 2007). As briefly reviewed by Davidge *et al.* (1987), experiments on ovariectomized heifers and cows have shown conflicting results about the influence of progesterone priming on oestradiol-induced oestrous behaviour. Although several studies cited by Davidge *et al.* (1987) have shown that oestrogen alone is sufficient to induce oestrus in ovariectomized heifers and cows, these studies did not attempt to determine the effect of progesterone on modifying the behavioural response. In their own study on ovariectomized cows, designed to test the hypothesis that progesterone before oestradiol-induced oestrus would have a regulating effect on the occurrence and intensity of oestrous behaviour, Davidge *et al.* (1987) found an inhibitory effect of progesterone. According to these authors, this unexpected result indicates that progesterone may not have the same effect on ovariectomized and intact cows.

In intact dairy cows, the importance of progesterone priming for the full display of oestrous behaviour is supported by the complete absence or short duration and low intensity of oestrous behaviour just before the first postpartum ovulation, an ovulation that is not preceded by a period of elevated progesterone blood levels (for references see Rhodes *et al.*, 2003). Failure to display behavioural oestrus just before the first *postpartum* ovulation may be explained by the induction of a refractory state of critical brain areas by the high oestradiol concentrations towards the end of pregnancy. It has been proposed that progesterone can undo this refractory state (Kyle *et al.*, 1992). It may be hypothesized that in cycling cows the elevated oestradiol concentrations during the follicular phase induces a similar refractory state that must be reset by the subsequent elevated progesterone concentrations during the luteal phase. Support for a significant influence of the level of circulating progesterone on the intensity of oestrous behaviour expression comes from a study on the oestrous behaviour of Mithun (*Bos frontalis*) cows (Dhali *et al.*, 2006). In these cows, bulls are absolutely needed to detect oestrus. The only visible signs of oestrus are reddening and swelling of the vulva, restlessness and standing to be mounted by a bull. Several other typical behavioural signs of oestrus expressed in *Bos taurus* cows, including cow–cow interactions, are not shown by Mithun cows. These cows have preovulatory oestradiol levels that are actually slightly higher than those found in *B. taurus* cows. However, their peak progesterone concentrations during the oestrous cycle are on average <3 ng/ml, whereas in Holstein dairy cows the average concentrations reported in a number of recent studies were in the

range of 6 to 8 ng/ml (Meier *et al.*, 2009; Herzog *et al.*, 2010; Starbuck and Mann, 2010). As in *B. taurus* cows, the lowest progesterone concentrations in Mithun cows (<0.5 ng/ml) were found on the day of oestrus (standing to be mounted by a bull). According to Dhali *et al.* (2006), the relatively weak expression of behavioural oestrus in Mithun cows is most probably because of the overall low progesterone concentrations throughout the oestrous cycle. Considering this, the Mithun cow seems to be a suitable animal model to study the role of progesterone in the expression of oestrous behaviour. It could be of interest to study the effect of progesterone supplementation of Mithun cows during the luteal phase of the cycle on subsequent expression of the various oestrous signs normally observed in *B. taurus* cows.

It has been suggested that the decline of the blood progesterone concentration following luteal regression may affect the expression of oestrous behaviour in domestic ruminants in ways other than through its effect on follicle growth and therefore on oestradiol synthesis (Fabre-Nys and Gelez, 2007). Experiments on ovariectomized French-Alpine goats that were sequentially treated with progesterone and oestrogen have shown that the decline in circulating progesterone concentrations before the rise of oestradiol may be more important than just the presence or absence of progesterone. In these goats, optimal expression of oestrous behaviour resulted when progesterone concentrations were maintained at luteal phase levels and then permitted to decline in a similar pattern as the decline which normally occurs during luteolysis (Katz, 2007). Irrespective of the kind of facilitating action, progesterone also inhibits the expression of oestrous behaviour in most species if its level is elevated directly before or during oestrus. According to Fabre-Nys and Gelez (2007), the elevated progesterone concentrations in pregnant female ruminants explain why they rarely display oestrous behaviour. In cattle, it is observed that some pregnant cows do show oestrous behaviour with such intensity that they would be considered in oestrus (Dijkhuizen and Van Eerdenburg, 1997). However, this does not preclude an inhibitory effect of progesterone on oestrous behaviour in cyclic cows, as the progesterone responsiveness and the behaviour-related neurophysiological mechanisms in the brain might be different in pregnant cows from that in cyclic cows.

It is assumed that the decreasing duration and intensity of behavioural oestrus over the past 50 years has been accompanied by a concomitant decrease in the blood concentrations of the ovarian steroids oestradiol and progesterone (Wiltbank *et al.*, 2006). An increased metabolic clearance of ovarian steroids has been suggested as a cause of lower blood concentrations of these steroids in high-producing cows. On the basis of the results of experiments on lactating and non-lactating cows on different feeding levels, Sangsritavang *et al.* (2002) concluded that the blood flow in the liver of high-producing dairy cows, which are at a continuous high plane of nutrition, may be chronically elevated, resulting in an increased metabolic clearance rate of oestradiol and progesterone. Further evidence in support of this conclusion comes from studies on Holstein cows (Rabiee *et al.*, 2001),

Zebu cows (Martins *et al.*, 2008) and ewes (Parr *et al.*, 1993a, and 1993b). In comparison with a group of Holstein cows on a restricted pasture diet, Rabiee *et al.* (2001) found in a comparable group of cows fed the same pasture diet *ad libitum* an increased excretion rate of progesterone to the faeces in association with a decreased concentration of peripheral progesterone. In a study involving Zebu cows with a low or a high feed intake, Martins *et al.* (2008) found no difference in the maximum diameter of the ovulatory follicle between the two groups, despite a lower preovulatory oestradiol surge in the cows with a high feed intake. This indicates a higher metabolism of oestradiol in the cows with a high feed intake. Furthermore, those authors reported no difference between the groups in progesterone concentration on day 7 after ovulation. Parr *et al.* (1993a and 1993b) studied the metabolic clearance rate of progesterone in ovariectomized ewes fed different levels of the same ration. They showed a positive relationship between feeding level and the blood flow through the intestines and to the liver, as well as an increased metabolic clearance of progesterone with increasing intestinal and hepatic blood flow. Parr *et al.* (1993b) concluded that the association between the level of feed intake and the chronic rate of blood flow to the liver, coupled with the high efficiency at which the liver metabolizes progesterone, favours the hypothesis that the inverse relationship between the level of feed intake and concentration of progesterone in peripheral plasma is controlled by the rate of splanchnic blood flow. Further suggestive evidence for a higher rate of metabolism of ovarian steroids because of high milk yield and the associated increased feed intake in cows comes from the study by Sartori *et al.* (2004) in which ovarian function and circulating ovarian steroids during oestrous cycles were compared between Holstein heifers and lactating dairy cows. In that study, the maximal serum oestradiol concentration preceding ovulation was found to be lower in cows than in heifers, despite the larger ovulatory follicles in cows. The maximal serum progesterone concentration too was lower for cows, whereas the maximal volume of the luteal tissue was lower for heifers. Other explanations for the relatively low blood concentrations of ovarian steroids in high-producing cows may be a lower steroidogenic activity of their ovarian follicles and corpora lutea (Wiltbank *et al.*, 2006) or the higher proportion of circulating ovarian steroids passing from blood into milk. The latter possibility has received little attention in literature. According to Erb *et al.* (1977), the amount of oestrogen secreted in milk is relatively small in comparison with that metabolized and excreted in urine and faeces. However, in high milk-producing cows, the amount of ovarian steroids secreted in milk may be biologically significant. For example, Lopez *et al.* (2002) reported the average oestradiol levels in plasma and milk to be ~15 and 5 pg/ml, respectively in high-producing primiparous Holstein cows at oestrus in early lactation. On the basis of the latter value, cows producing 40 to 50 kg milk/day would secrete about 200 to 250 ng oestradiol per day in their milk just before oestrus.

Oestrogen signalling and gene expression in the brain in relation to female sexual behaviour

Oestradiol 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). As far as the genomic mechanisms are concerned, oestradiol regulates transcription in the neurons and glia by the activation of classic nuclear oestrogen receptors (ERs). The two ERs, ER $_{\alpha}$ and ER $_{\beta}$, are products of different genes. They bind oestradiol with equal affinity but have different tissue distributions (Kuiper *et al.*, 1997). The classic mode of genomic action of oestradiol through activation of ERs is complemented by alternative non-genomic actions, whereby oestradiol acts at the membrane or in the cytoplasm of neurons. These non-genomic actions of oestradiol are linked to the activation of different kinases and second messenger systems such as cyclic adenosine monophosphate (cAMP) and intracellular calcium (Ca $^{2+}$), and activation of other transcriptional regulators such as cAMP response element-binding protein (Cardona-Gómez *et al.*, 2003; Vasudevan *et al.*, 2005 and reviewed by Kelly and Qiu, 2010). The effects of oestradiol in the brain may also be mediated by the activation of growth factor signalling (Cardona-Gómez *et al.*, 2003). The molecular and functional interactions of oestradiol and IGF-I in the brain have been reviewed by Cardona-Gómez *et al.* (2003). Molecular interactions include reciprocal regulation of ERs and IGF-I receptors (IGF-IRs), adrenergic α -1 receptor expression and activation, as well as MAPK and PI3K/Akt signalling pathway activation. Functional interactions between IGF and ER pathways affect axonal and dendritic growth, oestrous cycle-associated synaptic plasticity, gonadotropin release/ovulation, and reproductive behaviour/sexual receptivity. Reproductive success of the dairy cow is linked to its body energy reserves (Roche, 2006), which may be compromised, especially during early and peak lactation periods. The metabolic responses in the cow to low-energy reserves involve signalling molecules and hormones (such as IGF-I and growth hormones) that are central not only to the somatotrophic axis that regulates the partitioning of energy/nutrients but also to the hypothalamic–pituitary–ovarian axis that regulates reproductive function (Chagas *et al.*, 2007; Garnsworthy *et al.*, 2008). Some examples of genes expressed in the cow brain that plays roles in feeding behaviour, metabolic rate and feed intake, and also found associated with oestrous behaviour, are described towards the end of the next section.

Cell nuclear actions of oestradiol in the ventromedial hypothalamic (VMH) neurons, the transcriptional consequences of oestradiol binding in those neurons and how those neurons then regulate neural circuits that lead to lordosis behaviour (i.e., sexual receptivity) has been partly elucidated in rodents (Pfaff *et al.*, 2011). Primarily on the basis of the results of research by Pfaff and co-workers on lordosis behaviour in rodents, it has been proposed that oestradiol-activated genes in the VMH are organized in five modules referred to as GAPPs modules (Mong and Pfaff, 2004; Pfaff, 2005).

Here, GAPPS stands for: **G**rowth of hypothalamic neurons; **A**mplification of the oestrogen effects by progesterone; **P**reparative behaviours; **P**ermissive actions on the sex behaviour circuitry; and **S**ynchronization of mating behaviour with ovulation. The oestradiol-dependent growth effects on the neurons (first module) occur before lordosis behaviour is displayed. It permits oestradiol-facilitated, behaviour-directing hypothalamic neurons a greater range of input/output connections and thus physiological power. This module depends on ER $_{\alpha}$ -activated gene transcription. The amplification of the oestradiol effect by progesterone (second module) depends on both ER $_{\alpha}$ - and ER $_{\beta}$ -activated expression of the progesterone receptor (PGR) gene. The third module, preparations for mating by preceding behaviours includes ER $_{\alpha}$ -dependent expression of the enkephalin and opioid receptor genes to cause partial analgesia, as well as ER $_{\alpha}$ - and ER $_{\beta}$ -dependent transcription of the oxytocin and oxytocin receptor genes to reduce anxiety and aggression, and to improve social recognition. Analgesia and reduced anxiety allow the female animal to engage in courtship and mating behaviour, despite the risk of pain it may cause. The fourth module, permissive actions by hypothalamic neurons for the rest of the lordosis circuit to operate, includes the ER $_{\alpha}$ -dependent increased transcription of the genes encoding the noradrenergic α -1b receptor and the muscarinic acetylcholine receptors. These receptors are important for noradrenergic and acetylcholinergic signalling, respectively. The increased expression of these receptors permits the neural circuit to activate lordosis behaviour. Finally, ER $_{\beta}$ -dependent transcription of the GnRH and GnRH receptor genes (fifth module) serves to synchronize mating behaviour with ovulation.

When considering the GAPPS modules in relation to the neuroendocrine regulation of oestrous behaviour in species other than rodents, it should be kept in mind that the Amplification module may be the most variable module among species. For example, a difference between rodents and ungulates is the occurrence of a progesterone peak during pro-oestrus in rodents but not in ungulates. The progesterone peak in rodents occurs after the oestradiol peak and coincides more or less with the preovulatory LH peak (Pawluski *et al.*, 2009). The actions of this preovulatory progesterone peak are essential for the proceptive components of female sexual behaviour in rodents and for amplifying the stimulatory effects of oestradiol on receptivity (reviewed by Levine *et al.*, 2001 and Blaustein, 2008). Another important difference between ungulates and several groups within the *Cricetidae* and *Muridae* rodent families is that in these rodent species, development of a functional corpus luteum does not occur spontaneously after ovulation, as it does in ungulates, but requires stimuli associated with copulation. Thus, in these rodents, there is no luteal phase with elevated progesterone concentrations between subsequent oestrus periods and the inter-oestrus intervals are only 4 to 7 days long (Conaway, 1971). From this, it should not be concluded that the preovulatory progesterone peak in rodents is only important for potentiating receptivity (i.e., lordosis behaviour) in species without a true luteal phase. For example, female

guinea pigs, which present 16-day-long oestrous cycles with a true luteal phase, have a preovulatory progesterone peak with a potentiating effect on lordosis behaviour (Delville and Blaustein, 1991).

Oestrogen signalling and gene expression in the brain in relation to the expression of oestrous behaviour in dairy cows

To the best of our knowledge, the expression and distribution of ER $_{\beta}$ in the brain of cattle has not yet been studied, and only limited information is available about the expression and distribution of ER $_{\alpha}$. Tanaka *et al.* (2003) studied the distribution and morphology of immunoreactive ER $_{\alpha}$ -containing cells in the brain of Holstein Friesian cattle (a 44-month-old dry cow, a 27-month-old cow that had calved 1 week before killing and a 3-month-old male calf). ER $_{\alpha}$ signals were detected in the preoptic area (POA) of the rostral forebrain and the medial basal hypothalamic area (MBH). In the POA, they were located from the rostral part of the medial POA at the level of the vascular organ of the lamina terminalis to the region adjacent to the bed nucleus of the stria terminalis. In the MBH, the ER $_{\alpha}$ signals were also detected in the arcuate nucleus and the periventricular and VMH nuclei. Tanaka *et al.* (2003) concluded that the distribution of ER $_{\alpha}$ in the bovine hypothalamus closely parallels that of oestrogen-sensitive neurons in other mammalian species such as rats, guinea pigs and sheep (for references see Tanaka *et al.*, 2003). Changes in the distribution of immunoreactive ER $_{\alpha}$ in the hypothalamic area during the oestrous cycle of lactating dairy cows have been studied by Van Eerdenburg *et al.* (2000). During the luteal phase, ER $_{\alpha}$ immunoreactivity was found in most of the nuclei of cells in the bed nucleus of the stria terminalis, medial POA, ventromedial hypothalamus and arcuate nucleus. During oestrus and metoestrus, ER $_{\alpha}$ immunoreactivity could only be detected in a few cells in the caudal arcuate nucleus. According to Van Eerdenburg *et al.* (2000), this decline in ER number at oestrus–metoestrus might have developed in the herds of wild cattle with numerous cows and only a few bulls to downregulate receptive behaviour once ovulation has occurred and thus to avoid non-fertile matings.

Relatively little is known about the genomic regulation of oestrous behaviour 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 oestrous and luteal phase cows, respectively, has been measured, and the relation with oestrous behaviour of these cows was analysed (Kommadath *et al.*, 2010, 2011 and 2013; Kommadath, 2012). Oestrous behaviour was recorded in 28 healthy primiparous Holstein Friesian cows in several consecutive cycles between 30 days in milk (DIM) until they were killed. The time of killing varied between 77 and 139 DIM. The oestrous behaviour recorded in these cows was quantified as heat scores 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. Principal component analysis on individual cow cycle records indicated that there is a significant cow component in the heat score, that is, oestrous behaviour (heat score) to some extent was a consistent individual cow characteristic. Of the 28 cows, 14 were killed at the start of their oestrous cycle (day 0) and 14 around mid-cycle (day 12). Day 0 of the cycle was determined on the basis of ultrasonographical examinations of luteal regression and dominant follicle development, which were performed daily in the days before the expected day of oestrus (expected oestrus was based on the previous oestrus and previous cycle lengths), while using observations of vaginal mucous and oestrous behaviour as additional information. 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. In the rest of this section, we describe how the results of these studies can be integrated within the framework of the GAPPs modules in rodents described in the previous section (see Table 3). The names of the gene symbols used here are given in Supplementary Table S1.

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 oestradial-dependent outgrowth of hypothalamic neurons. This 'growth' may also apply to other brain areas (amygdala and hippocampus) and the anterior pituitary, as genes related to synaptic plasticity were not limited to the hypothalamic area. Several 'immune-related' genes found in the association studies, such as *CTLA4*, *IL1RL1*, *MARCO*, *FCRLA*, *IL33*, *CCL26* and *CXCL10*, may facilitate remodelling of synaptic networks. It has been shown that immunoglobulin superfamily proteins play important roles in brain developmental processes and the functioning of neuronal networks in adulthood because they provide the ideal structure for protein–protein interactions and thus cell–cell interactions (Rougon and Hobert, 2003). In addition, other genes can be grouped in the Growth module. For instance, analysis of gene co-expression networks between the brain areas and anterior pituitary (Kommadath *et al.*, 2013) revealed gene clusters that correlated with oestrous behaviour, of which a number of hub genes have been reported to have functions related to neuronal growth or plasticity: *NEFL* (Terry-Lorenzo *et al.*, 2000), *NDRG2* (Takahashi *et al.*, 2005), *THY1* (Rege and Hagood, 2006) and *GAP43* (Gispén *et al.*, 1991). The gene *NEFL*, for example, regulates the dephosphorylation of phosphoproteins implicated in synaptic plasticity. Furthermore, several ribosomal genes associated with oestrous behaviour in the association studies (*RPL24*; Kommadath *et al.*, 2011) or the co-expression studies (*RPL14*, *RPL18*, *RPS11*, *RPS18*; Kommadath *et al.*, 2013) across several brain areas. The increased synthesis of ribosomal RNA in the ventromedial hypothalamus has been reported to be one of the early effects of oestrogen administered subcutaneously in ovariectomized rats and represents a primary event in the activation of neuronal cells and neuronal pathways involved

in female reproductive behaviour (Jones *et al.*, 1990). Oestrogen-driven expansion of dendrites and synapses follows from the stimulation of synthesis of ribosomal RNA (Mong and Pfaff, 2004; Pfaff, 2005).

The second module in GAPPs is the Amplification of the oestrogen effect by progesterone, mediated by the nuclear PGR. In cattle, the expression of *PGR* in the anterior pituitary was found to be upregulated on day 0 (oestrous phase) compared with day 12 (luteal phase) of the oestrous cycle (Kommadath, 2012). The expression of *PGR* was not found to be associated with the heat score of the cows. Furthermore, in cattle, progesterone levels in cows remain low during oestrus. Nevertheless, an increased expression of *PGR* on day 0 could suggest a relation of the *PGR* with oestrous behaviour, perhaps via ligand-independent pathways (reviewed by Mani and Blaustein, 2012).

Several genes can also be identified that may be grouped within the Preparation module. For instance, the expression of oxytocin and arginine vasopressin genes in several brain areas of cows were associated with oestrous behaviour (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 behaviour and partner bonding (Leng *et al.*, 2008). In the presence of oestrogen, oxytocin exerts an anxiolytic effect, mediated by increases in oxytocin-binding density in the lateral septum, thereby favouring courtship and mating (McCarthy *et al.*, 1997; Mong and Pfaff, 2004). Similar to oxytocin, vasopressin is associated with sexual behaviour and bonding and its expression is under the control of oestrogen and progesterone (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 *POMC*, *MCHR1*, *CCK*, *DRD2*, *HTR2A* and *GABRA6*, whose expression levels in at least one of the brain areas are associated with oestrous behaviour 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 behaviour, 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 behaviour and gonadal function (Fabre-Nys, 1998). In ewes, dopamine-mediated D2 receptor (*DRD2*) signalling 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 oestrous 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 ewes are in oestrus (Fabre-Nys *et al.*, 1997). Studies on female rats and

Table 3 Oestrous behaviour-associated genes and processes in dairy cows grouped in the GAPPS modules described for female sexual behaviour in rodents (Mong and Pfaff, 2004)

GAPPS module	Corresponding genes and processes in cows ¹
Growth Increase in the input/output connections for behaviour-directing hypothalamic neurons or synaptic plasticity	<p>Immune-related genes <i>CTLA4</i> (\cup in $AM_{(0)}$, \cap in $VH_{(0)}$, \cup in $VH_{(12)}$) <i>IL1RL1</i> (\cap in $AM_{(12)}$) <i>MARCO</i> (\cap in $VH_{(0)}$, $-$ in $VH_{(0+12)}$)</p> <p>Neurotransmitter receptors <i>CHRM1</i> (\cup in $DH_{(12)}$) <i>CHRM3</i> (\cap in $VH_{(12)}$) <i>CHRNA5</i> (\cup in $AM_{(0)}$)</p> <p>Ribosomal genes <i>RPL14</i> (+ in AM : HC and HC : DH modules) <i>RPL18</i> (+ in AM : HC and HC : DH modules) <i>RPL24</i> (+ in $HC_{(0+12)}$, $-$ in AP : DH module) <i>RPS11</i> (+ in HC : DH, AM : DH and AM : HC modules) <i>RPS18</i> (+ in HC : DH and AM : HC modules)</p> <p>Other genes <i>NEFL</i> (+ in AM : DH and AP : DH modules) <i>NDRG2</i> (\cap in $AP_{(0)}$, + in AP : DH module) <i>THY1</i> (+ in AP : DH module)</p>
Amplification Amplification of oestrogen effect by progesterone mediated by progesterone receptor	<i>PGR</i> (upregulated in $AP_{(0 \text{ v. } 12)}$)
Preparation Preparation for mating	<p>Female sexual receptivity <i>OXT</i> ($-$ in $HC_{(0)}$, \cap in $DH_{(0)}$, $-$ in $DH_{(12)}$, + in AP : DH module) <i>AVP</i> ($-$ in $AM_{(0)}$, $-$ in $HC_{(0)}$, \cup in $DH_{(12)}$, + in AP : DH module) <i>HTR2A</i> (\cap in $VH_{(12)}$) <i>DRD2</i> ($-$ in $DH_{(0+12)}$) <i>GABRA6</i> (\cap in $AM_{(0)}$)</p> <p>Anxiolytic effect <i>OXT</i> ($-$ in $HC_{(0)}$, \cap in $DH_{(0)}$, $-$ in $DH_{(12)}$, + in AP : DH module) <i>TTR</i> (\cap in $HC_{(0)}$, \cup in $DH_{(0)}$, \cup in $DH_{(12)}$, \cup in $AM_{(12)}$) <i>KCNN2</i> (\cap in $AM_{(0)}$)</p> <p>Altered feeding motivation and mood <i>POMC</i> (\cap in $HC_{(0)}$, + in HC : DH module) <i>MCHR1</i> (\cup in $AM_{(0)}$, + in HC : DH module) <i>MOBP</i> (\cap in $AP_{(0)}$, $-$ in AP : DH module) <i>LTA4H</i> (+ in $AM_{(0)}$)</p>
Permission Permissive actions by hypothalamic neurons for the mating behaviour to occur	<p>Arousal, activation of protein kinases and release of Ca^{2+} <i>CHRM1</i> (\cup in $DH_{(12)}$) <i>CHRM3</i> (\cap in $VH_{(12)}$) <i>CHRNA5</i> (\cup in $AM_{(0)}$) <i>PLCB2</i> ($-$ in $AM_{(0)}$) <i>ITPKA</i> (\cap in $DH_{(12)}$, \cap in $VH_{(12)}$)</p>
Synchronization Synchronize mating behaviour with ovulation	<p>Prostaglandin regulators <i>PTGDS</i> (\cup in $AP_{(12)}$) <i>PTGIS</i> (\cup in $AM_{(0+12)}$) <i>PTGFR</i> (+ in $HC_{(12)}$, $-$ in AP : DH module)</p>

¹The relation of a gene with oestrus is described in brackets next to the gene name. Symbols '+', '-', ' \cup ' and ' \cap ' denote that a positive linear, a negative linear, a positive quadratic or a negative quadratic association, respectively, was found between the gene expression level in a brain area and the heat score of the cows (see details in Kommadath *et al.*, 2011). The brain areas are amygdala (AM), hippocampus (HC), dorsal hypothalamus (DH), ventral hypothalamus (VH) and anterior pituitary (AP). Subscripts $_{(0)}$, $_{(12)}$ or $_{(0+12)}$ denote that the association was found in cows killed in the oestrous phase (day 0), or in the luteal phase (day 12) or when considering all cows. If a gene is the member of a 'consensus module' (co-expressed genes in a pair of brain areas) whose representative expression level had a positive (+) or negative (-) association with oestrous behaviour, this is indicated as e.g. '(+ in AP : DH module)' (see details in Kommadath *et al.*, 2013). Data for *PGR* are from Kommadath, 2012.

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, *TTR*, *MOBP*, *LTA4H* and *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).

A number of oestrous behaviour score-associated genes (Kommadath *et al.*, 2010 and 2011) may be grouped in the fourth GAPPS module – Permission. For instance, the association between oestrous behaviour 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 Ca^{2+} via the phospholipase C – inositol 1,4,5-trisphosphate signalling 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 oestrous behaviour scores. These findings can be explained based on the hypothesis put forward by Kow and Pfaff (2004) that the membrane actions of oestrogen can modulate the genomic actions of oestrogen and that this transcriptional potentiation is mediated via signalling pathways requiring the activation of certain protein kinases and increased intracellular Ca^{2+} .

Finally, the heat score-associated genes *PTGDS*, *PTGIS* and *PTGFR* (Kommadath *et al.*, 2011 and 2013) may be grouped in the last GAPPS module (Synchronization). These genes regulate prostaglandin functioning. Prostaglandins are known to be under the influence of oestrogen (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).

Conclusion

Current research findings indicate that the decreased expression of oestrous behaviour seen in high-producing dairy cows is related to decreased blood concentrations of ovarian steroids, 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 oestrous behaviour in dairy cows may contribute to the development of tools and management strategies to improve oestrous behaviour detection and therewith reproductive efficiency. In addition,

this understanding may facilitate the development of a (genomic) selection strategy for improving the trait of oestrous behaviour expression, thereby improving reproductive efficiency in high-producing dairy cows. A firm base of understanding of the ovarian steroid-driven central genomic regulation of female sexual behaviour exists in rodents. The recent studies on cattle reviewed in this paper have now contributed first steps towards such understanding in cattle and point to a functional clustering of oestrous behaviour-associated genes in cattle within the GAPPS modules described earlier for female sexual behaviour in rodents.

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Supplementary material

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References

- Allrich RD 1994. Endocrine and neural control of estrus in dairy cows. *Journal of Dairy Science* 77, 2738–2744.
- Amateau SK and McCarthy MM 2002. A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin- E_2 . *Journal of Neuroscience* 22, 8586–8596.
- Billups D, Billups B, Challiss RAJ and Nahorski SR 2006. Modulation of G $_q$ -protein-coupled inositol trisphosphate and Ca^{2+} signaling by the membrane potential. *Journal of Neuroscience* 26, 9983–9995.
- Blaustein JD 2008. Neuroendocrine regulation of feminine sexual behavior: lessons from rodent models and thoughts about humans. *Annual Review of Psychology* 59, 93–118.
- Caldwell HK and Albers HE 2002. The effects of serotonin agonists on the hypothalamic regulation of sexual receptivity in Syrian hamsters. *Hormones and Behavior* 42, 78–84.
- Cardona-Gómez GP, Mendez P, DonCarlos LL, Azcoitia I and Garcia-Segura LM 2003. Interactions of estrogen and insulin-like growth factor-I in the brain: molecular mechanisms and functional implications. *Journal of Steroid Biochemistry and Molecular Biology* 83, 211–217.
- Chagas LM, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin GB, Meier S, Rhodes FM, Roche JR, Thatcher WW and Webb R 2007. Invited review: new perspectives on the roles of nutrition and metabolic priorities in the sub-fertility of high-producing dairy cows. *Journal of Dairy Science* 90, 4022–4032.
- Clasadonte J, Poulain P, Hanchate NK, Corfas G, Ojeda SR and Prevot V 2011. Prostaglandin E_2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation. *Proceedings of the National Academy of Sciences of the United States of America* 108, 16104–16109.
- Conaway CH 1971. Ecological adaptation and mammalian reproduction. *Biology of Reproduction* 4, 239–247.
- Curley JP and Keverne EB 2005. Genes, brains and mammalian social bonds. *Trends in Ecology and Evolution* 20, 561–567.

- Davidge ST, Wiebold JL, Senger PL and Hillers JK 1987. Influence of varying levels of blood progesterone upon estrous behavior in cattle. *Journal of Animal Science* 64, 126–132.
- Delville Y and Blaustein JD 1991. A site for estradiol priming of progesterone-facilitated sexual receptivity in the ventrolateral hypothalamus of female guinea pigs. *Brain Research* 559, 191–199.
- Dhali A, Mishra DP, Karunakaran M, Mech A and Rajkhowa C 2006. Influence of plasma estradiol 17- β and progesterone levels on estrous behaviour in Mithun (*Bos frontalis*). *Applied Animal Behaviour Science* 98, 1–10.
- Dijkhuizen TJ and Van Eerdenburg FJCM 1997. Behavioural signs of oestrus during pregnancy in lactating dairy cows. *Veterinary Quarterly* 119, 194–196.
- Diskin MG and Sreenan JM 2000. Expression and detection of oestrus in cattle. *Reproduction Nutrition Development* 40, 481–491.
- Dobson H, Walker SL, Morris MJ, Routly JE and Smith RF 2008. Why is it getting more difficult to successfully artificially inseminate dairy cows? *Animal* 2, 1104–1111.
- Donaldson ZR and Young LJ 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* 322, 900–904.
- Dransfield MBG, Nebel RL, Pearson RE and Warnick LD 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. *Journal of Dairy Science* 81, 1874–1882.
- Erb RE, Chew BP and Keller HF 1977. Relative concentrations of estrogen and progesterone in milk and blood, and excretion of estrogen in urine. *Journal of Animal Science* 46, 617–626.
- Esslemont RJ, Glencross RG, Bryant MJ and Pope GS 1980. A quantitative study of pre-ovulatory behaviour in cattle (British Friesian heifers). *Applied Animal Ethology* 6, 1–17.
- Fabre-Nys C 1998. Steroid control of monoamines in relation to sexual behaviour. *Reviews of Reproduction* 3, 31–41.
- Fabre-Nys C and Gelez H 2007. Sexual behavior in ewes and other domestic ruminants. *Hormones and Behavior* 52, 18–25.
- Fabre-Nys C, Ohkura S and Kendrick KM 1997. Male faces and odours evoke differential patterns of neurochemical release in the mediobasal hypothalamus of the ewe during oestrus: an insight into sexual motivation? *European Journal of Neuroscience* 9, 1666–1677.
- Fabre-Nys C, Chesneau D, De La Riva C, Hinton MR, Locatelli A, Ohkura S and Kendrick KM 2003. Biphasic role of dopamine on female sexual behaviour via D2 receptors in the mediobasal hypothalamus. *Neuropharmacology* 44, 354–366.
- Firk R, Stamer E, Junge W and Krieter J 2002. Automation of oestrus detection in dairy cows: a review. *Livestock Production Science* 75, 219–232.
- García E, Hultgren J, Fällman P, Geust J, Algers B, Stilwell G, Gunnarsson S and Rodriguez-Martinez H 2011. Oestrous intensity is positively associated with reproductive outcome in high-producing dairy cows. *Livestock Science* 139, 191–195.
- Garnsworthy PC, Sinclair KD and Webb R 2008. Integration of physiological mechanisms that influence fertility in dairy cows. *Animal* 2, 1144–1152.
- Gilmore HS, Young FJ, Patterson DC, Wylie ARG, Law RA, Kilpatrick DJ, Elliott CT and Mayne CS 2011. An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior of high-yielding Holstein-Friesian dairy cows. *Journal of Dairy Science* 94, 3510–3526.
- Gispén WH, Nielander HB, De Graan PNE, Oestreicher AB, Schrama LH and Schotman P 1991. Role of the growth-associated protein B-50/GAP-43 in neuronal plasticity. *Molecular Neurobiology* 5, 61–85.
- Herzog K, Brockhan-Lüdemann M, Kasse M, Beindorff N, Paul V, Niemann H and Bollwein H 2010. Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. *Theriogenology* 73, 691–697.
- Holman A, Thompson J, Routly JE, Cameron J, Jones DN, Grove-White D, Smith RF and Dobson H 2011. Comparison of oestrus detection methods in dairy cattle. *The Veterinary Record* 169, 47.
- Hurnik JF, King GJ and Robertson HA 1975. Estrous and related behaviour in postpartum Holstein cows. *Applied Animal Ethology* 2, 55–68.
- Inchaisri C, Jorritsma R, Vos PLAM, Van der Weijden GC and Hogeveen H 2010. Economic consequences of reproductive performance in dairy cattle. *Theriogenology* 74, 835–846.
- Jasoni CL, Todman MG, Han SK and Herbison AE 2005. Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of the mouse. *Neuroendocrinology* 82, 320–328.
- Jones KJ, Harrington CA, Chikaraishi DM and Pfaff DW 1990. Steroid hormone regulation of ribosomal RNA in rat hypothalamus: early detection using *in situ* hybridization and precursor-product ribosomal DNA probes. *Journal of Neuroscience* 10, 1513–1521.
- Kalamatianos T, Kalló I, Goubillon ML and Coen CW 2004. Cellular expression of V_{1a} vasopressin receptor mRNA in the female rat preoptic area: effects of oestrogen. *Journal of Neuroendocrinology* 16, 525–533.
- Katz LS 2007. Sexual behavior of domesticated ruminants. *Hormones and Behavior* 52, 56–63.
- Kelly MJ and Qiu J 2010. Estrogen signaling in hypothalamic circuits controlling reproduction. *Brain Research* 1364, 44–52.
- Kommadath A 2012. Genomic regulation of oestrous behaviour in dairy cattle. Thesis PhD, Wageningen University, The Netherlands.
- Kommadath A, Te Pas MFW and Smits MA 2013. Gene co-expression network analysis identifies genes and biological processes shared among anterior pituitary and brain areas that affect estrous behavior in dairy cow. *Journal of Dairy Science* 96, 2583–2595.
- Kommadath A, Woelders H, Beerda B, Mulder HA, De Wit AAC, Veerkamp RF, Te Pas MFW and Smits MA 2011. Gene expression patterns in four brain areas associate with quantitative measure of estrous behavior in dairy cows. *BMC Genomics* 12, 200.
- Kommadath A, Mulder HA, De Wit AAC, Woelders H, Smits MA, Beerda B, Veerkamp RF, Frijters ACJ and Te Pas MFW 2010. Gene expression patterns in anterior pituitary associated with quantitative measure of oestrous behaviour in dairy cows. *Animal* 4, 1297–1307.
- Kow LM and Pfaff DW 2004. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. *Proceedings of the National Academy of Sciences of the United States of America* 101, 12354–12357.
- Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S and Gustafsson JÅ 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 138, 863–870.
- Kyle SD, Callahan CJ and Allrich RD 1992. Effect of progesterone on the expression of estrus at the first postpartum ovulation in dairy cattle. *Journal of Dairy Science* 75, 1456–1460.
- Leng G, Meddle SL and Douglas AJ 2008. Oxytocin and the maternal brain. *Current Opinion in Pharmacology* 8, 731–734.
- Levine JE, Chapel PE, Schneider JS, Sleiter NC and Szabo M 2001. Progesterone receptors as neuroendocrine integrators. *Frontiers in Neuroendocrinology* 22, 69–106.
- Lopez H, Bunch TD and Shipka MP 2002. Estrogen concentrations in milk at estrus and ovulation in dairy cows. *Animal Reproduction Science* 72, 37–46.
- Lopez H, Satter LD and Wiltbank MC 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. *Animal Reproduction Science* 81, 209–223.
- Lyimo ZC, Nielen M, Ouweltjes W, Kruij TAM and Van Eerdenburg FJCM 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology* 53, 1783–1795.
- Mani SK and Blaustein JD 2012. Neural progesterin receptors and female sexual behavior. *Neuroendocrinology* 96, 152–161.
- Marsh DJ, Weingarth DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, Guan X-M, Jiang MM, Feng Y, Camacho RE, Shen Z, Frazier EG, Yu H, Metzger JM, Kuca SJ, Shearman LP, Gopal-Truter S, MacNeil DJ, Strack AM, MacIntyre DE, Van der Ploeg LHT and Qian S 2002. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proceedings of the National Academy of Sciences of the United States of America* 99, 3240–3245.
- Martins AC, Mollo MR, Bastos MR, Guardieiro MM and Sartori R 2008. Serum hormone concentrations of zebu cows under low and high feed intake. *Pesquisa Agropecuária Brasileira* 43, 243–247.
- McCarthy MM, McDonald CH, Brooks PJ and Goldman D 1997. An anxiolytic action of oxytocin is enhanced by estrogen in the mouse. *Physiology & Behavior* 60, 1209–1215.
- Meier S, Roche JR, Kolver ES, Verkerk GA and Boston RC 2009. Comparing subpopulations of plasma progesterone using cluster analyses. *Journal of Dairy Science* 92, 1460–1468.
- Millington GWM 2007. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. *Nutrition & Metabolism* 4, 18.

- Mitra R, Ferguson D and Sapolsky RM 2009. SK2 potassium channel over-expression in basolateral amygdala reduces anxiety, stress-induced corticosterone and dendritic arborization. *Molecular Psychiatry* 14, 847–855.
- Mong JA and Pfaff DW 2004. Hormonal symphony: steroid orchestration of gene modules for sociosexual behaviors. *Molecular Psychiatry* 9, 550–556.
- Parr RA, Davis IF, Miles MA and Squires TJ 1993a. Feed intake affects metabolic clearance rate of progesterone in sheep. *Research in Veterinary Science* 55, 306–310.
- Parr RA, Davis IF, Miles MA and Squires TJ 1993b. Liver blood flow and metabolic clearance rate of progesterone in sheep. *Research in Veterinary Science* 55, 311–316.
- Patisaul HB, Scordalakes EM, Young LJ and Rissman EF 2003. Oxytocin, but not oxytocin receptor, is regulated by oestrogen receptor β in the female mouse hypothalamus. *Journal of Neuroendocrinology* 15, 787–793.
- Pawluski JL, Brummelte S, Barha CK, Crozier TM and Galea LAM 2009. Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. *Frontiers in Neuroendocrinology* 30, 343–357.
- Pfaff D 2005. Hormone-driven mechanisms in the central nervous system facilitate the analysis of mammalian behaviours. *Journal of Endocrinology* 184, 447–453.
- Pfaff D, Waters E, Khan Q, Zhang X and Numan M 2011. Estrogen receptor-initiated mechanisms causal to mammalian reproductive behaviors. *Endocrinology* 152, 1209–1217.
- Rabiee AR, Macmillan KL and Schwarzenberger F 2001. The effect of level of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows. *Animal Reproduction Science* 67, 205–214.
- Reames PS, Hatler TB, Hayes SH, Ray DL and Silvia WJ 2011. Differential regulation of estrous behavior and luteinizing hormone secretion by estradiol-17 β in ovariectomized dairy cows. *Theriogenology* 75, 233–240.
- Rege TA and Hagood JS 2006. Thy-1 as a regulator of cell-cell and cell-matrix interactions in axon regeneration, apoptosis, adhesion, migration, cancer, and fibrosis. *FASEB Journal* 20, 1045–1054.
- Rex A, Marsden CA and Fink H 1997. Cortical 5-HT-CCK interactions and anxiety-related behaviour of guinea-pigs: a microdialysis study. *Neuroscience Letters* 228, 79–82.
- Rhodes FM, McDougall S, Burke CR, Verkerk GA and MacMillan KL 2003. Treatment of cows with an extended postpartum anestrous interval. *Journal of Dairy Science* 86, 1876–1894.
- Roche JF 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. *Animal Reproduction Science* 96, 282–296.
- Roelofs J, López-Gatius F, Hunter RHF, Van Eerdenburg FJCM and Hanzen Ch 2010. When is a cow in estrus? Clinical and practical aspects. *Theriogenology* 74, 327–344.
- Roelofs JB, Van Eerdenburg FJCM, Soede NM and Kemp B 2005. Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. *Theriogenology* 63, 1366–1377.
- Rougon G and Hobert O 2003. New insights into the diversity and function of neuronal immunoglobulin superfamily molecules. *The Annual Review of Neuroscience* 26, 207–238.
- Sangsrivavong S, Combs DK, Sartori R, Armentano LE and Wiltbank MC 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17 β in dairy cattle. *Journal of Dairy Science* 85, 2831–2842.
- Sartori R, Haughian JM, Shaver RD, Rosa GJM and Wiltbank MC 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *Journal of Dairy Science* 87, 905–920.
- Schober JM and Pfaff D 2007. The neurophysiology of sexual arousal. *Best Practice & Research Clinical Endocrinology & Metabolism* 21, 445–461.
- Sokolov BP 2007. Oligodendroglial abnormalities in schizophrenia, mood disorders and substance abuse. Comorbidity, shared traits, or molecular phenocopies? *The International Journal of Neuropsychopharmacology* 10, 547–555.
- Sousa JC, Grandela C, Fernández-Ruiz J, De Miguel R, De Sousa L, Magalhães AI, Saraiva MJ, Sousa N and Palha JA 2004. Transthyretin is involved in depression-like behaviour and exploratory activity. *Journal of Neurochemistry* 88, 1052–1058.
- Starbuck GR and Mann GE 2010. Differential effects of exogenous progesterone administration at different stages of the luteal phase on endogenous oestradiol concentration in cows. *Reproduction in Domestic Animals* 45, 283–286.
- Takahashi K, Yamada M, Ohata H, Honda K and Yamada M 2005. NdrG2 promotes neurite outgrowth of NGF-differentiated PC12 cells. *Neuroscience Letters* 388, 157–162.
- Tanaka T, Shiina T, Hayashi S, Okamura H, Kamomae H and Kaneda Y 2003. Estrogen receptor alpha expression in the medial preoptic area and the medial basal hypothalamus under different physiological conditions in cattle. *Journal of Reproduction and Development* 49, 55–60.
- Terry-Lorenzo RT, Inoue M, Connor JH, Haystead TAJ, Armbruster BN, Gupta RP, Oliver CJ and Shenolikar S 2000. Neurofilament-L is a protein phosphatase-1-binding protein associated with neuronal plasma membrane and post-synaptic density. *Journal of Biological Chemistry* 275, 2439–2446.
- Uhart M, McCaul ME, Oswald LM, Choi L and Wand GS 2004. *GABRA6* gene polymorphism and an attenuated stress response. *Molecular Psychiatry* 9, 998–1006.
- Uphouse L 2000. Female gonadal hormones, serotonin, and sexual receptivity. *Brain Research Reviews* 33, 242–257.
- Van Eerdenburg FJCM, Loeffler HSH and Van Vliet JH 1996. Detection of oestrus in dairy cows: a new approach to an old problem. *Veterinary Quarterly* 18, 52–54.
- Van Eerdenburg FJCM, Daemen IAJM, Van der Beek EM and Van Leeuwen FW 2000. Changes in estrogen-alpha receptor immunoreactivity during the estrous cycle in lactating dairy cattle. *Brain Research* 880, 219–223.
- Vasudevan N, Kow LM and Pfaff D 2005. Integration of steroid hormone initiated membrane action to genomic function in the brain. *Steroids* 70, 388–396.
- Walsh SW, Williams EJ and Evans ACO 2011. A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science* 123, 127–138.
- Wiltbank M, Lopez H, Sartori R, Sangsrivavong S and Gümen A 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology* 65, 17–29.
- Yoshida C and Nakao T 2005. Some characteristics of primary and secondary oestrous signs in high-producing dairy cows. *Reproduction in Domestic Animals* 40, 150–155.
- Zhao J, Quyyumi AA, Patel R, Zafari AM, Veledar E, Onufrak S, Shallenberger LH, Jones L and Vaccarino V 2009. Sex-specific association of depression and a haplotype in leukotriene A4 hydrolase gene. *Psychosomatic Medicine* 71, 691–696.